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Interactions corticales impliquées dans la production des mouvements de la main chez le singe capucin

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Cette thèse intitulée

Interactions corticales impliquées dans la production des mouvements de la main chez le singe capucin

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Résumé

Chez les primates, le raffinement des mouvements de la main est associé à l'apparition d'aires prémotrices corticales additionnelles. Chacune de ces aires prémotrices semble avoir une fonction spécialisée dans le contrôle moteur de la main, appuyant l'idée qu'elles sont apparues au cours de l'évolution afin de soutenir un répertoire comportemental accru. Afin de participer à l'exécution de ce vaste répertoire, il est suggéré que les aires prémotrices modulent les efférences du cortex moteur primaire (M1), une aire corticale jouant un rôle clé dans la production des mouvements volontaires. En effet, grâce à leurs nombreuses projections cortico-corticales vers M1 ainsi que leurs projections vers des structures sous-corticales qui sont également innervées par M1, les aires prémotrices se trouvent dans une position idéale pour moduler les efférences motrices de M1. Néanmoins, la contribution de ces projections anatomiques à la production des mouvements de la main demeure peu comprise. La fonction de ces projections est toutefois importante à investiguer afin de mieux comprendre les interactions corticales qui sous-tendent l'augmentation du répertoire des mouvements de la main chez les primates.

S'intégrant dans ce contexte de recherche, les expériences présentées dans cette thèse visent à caractériser les interactions corticales entre les aires prémotrices et M1 qui sont impliquées dans les mouvements de la main chez le singe capucin. Dans une première étude, les effets modulateurs du cortex prémoteur ventral (PMv) sur les efférences de M1 ont été investigués (**Chapitre I**). Dans une seconde étude, les effets modulateurs du cortex prémoteur dorsal (PMd) ont été étudiés et comparés à ceux de PMv (**Chapitre II**). Finalement, dans une troisième étude, les effets modulateurs de l'aire supplémentaire motrice (SMA) ont été examinés et comparés à ceux de PMv et de PMd (**Chapitre III**).

En résumé, les résultats présentés dans cette thèse offrent une nouvelle perspective quant aux interactions corticales liant les aires prémotrices à M1. Il est démontré que chaque aire prémotrice influence les efférences de M1 de manière unique. Ceci appuie l'idée que chaque aire prémotrice joue un rôle spécialisé dans le contrôle moteur de la main et est en mesure d'accomplir cette fonction, entre autres, à travers sa modulation des efférences motrices de M1. Ces résultats contribuent à une meilleure compréhension des interactions corticales qui sous-tendent le raffinement des mouvements de la main accompagnant l'évolution du système moteur.

Mots-clés: aires prémotrices, cortex moteur primaire, interactions, main, mouvement

Abstract

In primates, the refinement of hand movements is associated with the appearance of additional cortical premotor areas. Each of these premotor areas appears to have a specialized function in the motor control of the hand, supporting the idea that they have appeared during evolution to support an increased behavioral repertoire. In order to participate in the execution of this vast repertoire, it is suggested that the premotor areas modulate the motor outputs of the primary motor cortex (M1), a cortical area that plays a key role in the production of voluntary movements. Indeed, thanks to their numerous cortico-cortical projections to M1 as well as their projections to sub-cortical structures also innervated by M1, premotor areas are in an ideal position to modulate the motor outputs of M1. Nevertheless, the contribution of these anatomical projections to the production of hand movements is still unclear. The function of these projections, however, is important to investigate in order to better understand the cortical interactions that underlie the increased motor repertoire of primates.

As an integral part of this research context, the experiments presented in this thesis aim to characterize the cortical interactions between the premotor areas and M1 involved in hand movements in the capuchin monkey. In a first study, the modulatory effects of ventral premotor cortex (PMv) on M1 outputs were investigated (**Chapter I**). In a second study, the modulatory effects of the dorsal premotor cortex (PMd) were studied and compared to those of PMv (**Chapter II**). Lastly, in a third study, the modulatory effects of the supplementary motor area (SMA) were examined and compared to those of PMv and PMd (**Chapter III**).

In summary, the results presented in this thesis offer a new perspective on the cortical interactions linking the premotor areas to M1. It is shown that each premotor area influences the outputs of M1 in a unique way. This supports the idea that each premotor area plays a specialized role in the motor control of the hand and is able to accomplish this function, in part, through its modulation of M1 outputs. These results contribute to a better understanding of the cortical interactions that underlie the refinement of hand movements accompanying the evolution of the motor system.

Keywords: hand, interactions, movement, primary motor cortex, premotor areas

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AVC : accident vasculaire-cérébral

BMI: interfaces cerveau-machine

C: conditionnant

ccPAS: stimulation pairée associative cortico-corticale

CM: connexions cortico-motoneuronales

EMG: électromyographique

GABA : acide y-aminobutyrique

ICMS: microstimulation intracorticale

ISI: intervalle inter-stimulus

LFP : potentiel de champ local

LTD: potentialisation à long terme

LTD: potentialisation à long terme

M1: cortex moteur primaire

MCAo: middle cerebral artery occlusions

MEP: motor evoked potential

PMd: cortex prémoteur dorsal

PMv: cortex prémoteur ventral

PMRF: formation réticulée pontomédullaire

rTMS: stimulation magnétique transcrânienne répétitive

RST: voie réticulospinale

RuST: voie rubrospinale

S1: cortex sensoriel primaire

SMA: aire supplémentaire motrice

STDP: spike timing dependent plasticity

T: test

TMS: stimulation magnétique transcrânienne

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Chapitre 1 - Introduction

1. Contexte historique de la recherche sur le cortex moteur

Cette section présente l'émergence des idées actuelles concernant l'organisation motrice corticale en retraçant les moments clefs de la recherche sur le cortex moteur. D'abord, des études de stimulation électrique à la surface du cortex cérébral ont permis la découverte d'une région circonscrite consacrée à la fonction motrice: le cortex moteur. Puis, une exploration plus précise de cette région motrice à mener à sa division en de multiples sous-régions, formant un réseau moteur cortical complexe dont la principale fonction est le contrôle volontaire du mouvement.

1.1. La découverte du cortex moteur

Les connaissances actuelles sur l'organisation corticale motrice des primates tirent leur origine d'études classiques chez l'animal et chez l'humain démontrant qu'une stimulation électrique appliquée à la surface du cortex peut évoquer des mouvements localisés du côté contralatéral à l'hémisphère stimulé (Ferrier, 1874; Leyton and Sherrington, 1917; Penfield and Boldrey, 1937; Fritsch and Hitzig, 2009). À travers ces expériences, il a été constaté que les réponses motrices évoquées par la stimulation proviennent d'une région circonscrite du lobe frontal, tout juste antérieure au sillon central, dénommée cortex moteur. Certains de ces premiers travaux ont également mis en évidence l'existence d'une organisation somatotopique au sein du cortex moteur; où différents territoires corticaux régissent le mouvement de segments spécifiques du corps (Leyton and Sherrington, 1917; Penfield and Boldrey, 1937). Cette cartographie du corps sur la surface du cortex, nommée homoncule, est organisée de manière ordonnée avec, à ces extrêmes médial et latéral, la représentation de la jambe et de la bouche, respectivement. En outre, l'étendue du territoire dédiée à chaque partie du corps est proportionnelle à la complexité des mouvements que celle-ci peut effectuer et non pas à la taille de cette dernière. Par exemple, la taille du territoire occupé par la représentation de la main est disproportionnée par rapport à la taille de celle occupée par la représentation de la jambe ou du tronc. Cette organisation corticale confèrerait aux primates leur remarquable dextérité manuelle. Il est a noté que l'organisation très ordonnée des différentes représentations du corps proposée par ces études classiques a été réfutée par des études plus

récentes démontrant que le cortex moteur est plutôt organisé comme une mosaïque où certaines représentations du corps telles que celles de la main et de l'avant-bras se chevauchent partiellement (voir section 2.1). Ainsi, l'organisation du cortex moteur semble plus complexe qu'initialement estimé.

1.2. Les aire corticales motrices additionnelles

À l'origine, une seule région motrice corticale était reconnue. Cependant, au début du 20^{e} siècle, une nouvelle hypothèse soutenant l'existence de plusieurs zones motrices corticales a émergée. Cette idée a été introduite par une analyse cytoarchitectonique démontrant que le cortex moteur pouvait être séparé en deux régions: une région postérieure caractérisée par une population dense de cellules pyramidales géantes et une région antérieure caractérisée par l'absence de ces cellules (Campbell, 1905). Campbell spécula également que des différences fonctionnelles distinguaient ces deux régions motrices, avec la région postérieure dite « primaire » (cortex moteur primaire ou M1) contrôlant les paramètres simples du mouvement et la région antérieure contrôlant des paramètres d'ordre plus complexe. Par la suite, diverses expériences corroborèrent l'hypothèse de non-uniformité du cortex moteur (Broadmann, 1909; Vogt and Vogt, 1919) et Fulton popularisa le terme « prémoteur » pour caractériser la région antérieure à M1 sur la surface latérale et médiale du cortex (Fulton, 1935). Cependant, dans les années 50, certains chercheurs proposèrent une organisation alternative et rejetèrent l'existence d'un cortex prémoteur localisé sur la surface latérale du cortex (Penfield and Welch, 1951; Woolsey et al., 1952). Ceux-ci soutinrent que seulement la région antérieure à M1 située sur la surface médiale du cortex pouvait être considérée comme une deuxième région motrice corticale car ils y trouvèrent la seule carte du corps distincte de celle de M1. Ils appelèrent cette région M2 ou aire motrice supplémentaire (SMA). Le point de vue de Penfield et de Woolsey a fortement influencé le domaine. Cependant, avec l'avènement de techniques d'imagerie chez l'humain et de techniques plus invasives chez le primate non-humain, de nouvelles évidences ont convergées en faveur de la présence d'une région prémotrice sur la surface latérale du cortex (Roland et al., 1980a; Weinrich and Wise, 1982; Weinrich et al., 1984; Matelli et al., 1985; Gentilucci et al., 1988; Rizzolatti et al., 1988). Depuis, un consensus s'est dégagé: le contrôle moteur volontaire n'émane pas d'une seule région corticale, mais de multiples sous-régions qui incluent M1, deux aires prémotrices latérales: le cortex prémoteur ventral (PMv) et le cortex prémoteur dorsal (PMd) et plusieurs aires prémotrices médiales dont l'aire motrice

supplémentatire (SMA). Ces multiples régions motrices corticales seront discutées en détail dans la prochaine section.

2. Le réseau moteur cortical impliqué dans les mouvements de la main

Les primates sont dotés d'une grande dextérité manuelle et sont capables d'exécuter des mouvements volontaires extrêmement complexes. Ces capacités reposent sur un vaste réseau moteur cortical incluant le cortex moteur primaire (M1) et différentes aires prémotrices. La section suivante décrit les propriétés et fonctions de ces régions motrices corticales en mettant l'emphase sur le contrôle moteur de la main.

2.1 Le cortex moteur primaire (M1)

À la suite des études classiques de stimulation de surface (Penfield and Boldrey, 1937; Woolsey et al., 1952), des techniques plus invasives ont commencé à être adoptées afin d'étudier le cortex moteur de manière plus détaillée, particulièrement à l'intérieur de la représentation de la main et du bras. Asanuma et collègues ont été les premiers à cartographier le cortex moteur avec la technique de microstimulation intracorticale (ICMS) chez l'animal sous sédation (Asanuma and Sakata, 1967; Asanuma and Ward, 1971; Asanuma and Rosen, 1972). Cette technique utilise une microélectrode insérée dans le cortex afin de stimuler une petite sphère de tissu entourant la pointe de l'électrode. En employant une stimulation de basse intensité (μA vs mA), l'ICMS a l'avantage d'avoir une résolution spatiale plus élevée que la stimulation de surface (μm vs mm) (Asanuma and Sakata, 1967). Ces travaux ont mené Asanuma à suggérer la présence d'une organisation somatotopique musculaire très stricte dans le cortex moteur, où chaque muscle individuel de la main serait représenté au sein d'une région circonscrite (Asanuma, 1975). Cette hypothèse a toutefois été invalidée par des études anatomiques démontrant que les terminaisons axonales d'un neurone corticospinal peuvent cibler des motoneurones innervant différents muscles de la main et que les neurones corticospinaux innervant les motoneurones d'un muscle individuel occupent un vaste territoire à l'intérieur de M1 (Shinoda et al., 1981; Lawrence et al., 1985; Rathelot and Strick, 2006). De plus, des études électrophysiologiques chez le macaque éveillé utilisant une variante de l'ICMS, le *stimulus-triggered averaging*, ainsi que le *spike-triggered averaging*, ont démontré que la stimulation d'un site dans M1 ainsi que l'activité d'un neurone individuel dans M1 peut influencer l'activité de plusieurs muscles de l'avant-bras (Cheney and Fetz, 1985) ou de la main (Buys et al., 1986). Ainsi, les muscles ne semblent généralement pas représentés de manière isolée dans M1. À l'opposé, chaque emplacement et même chaque neurone corticospinal dans M1 semble

évoquer un patron complexe d'activité à travers un ensemble de muscles. Parallèlement, les neurones corticospinaux de M1 contrôlant un muscle spécifique sont largement répartis au sein de M1. Des études extensives de cartographie par ICMS chez le macaque sont également venues appuyer ces conclusions. Par exemple, il a été démontré qu'au niveau de la représentation du bras de M1, la représentation des muscles distaux (main et poignet) et proximaux (coude et épaule) se superposent largement (Gould et al., 1986; Huntley and Jones, 1991; Donoghue et al., 1992). Plus récemment, Park et al., (2001) ont discerner trois zones dans la représentation du bras de M1 chez le macaque: une zone centrale au niveau du mur antérieur du sillon central qui évoque uniquement des réponses dans les muscles distaux, une zone de transition qui évoque des réponses dans les muscles distaux et proximaux et une zone englobante qui évoque uniquement des réponses dans les muscles proximaux. Il est proposé que le chevauchement local à l'intérieur de la représentation du bras permet de coordonner les mouvements qui impliquent plusieurs articulations, comme les mouvements d'atteinte et de saisie d'objets (Park et al., 2001). Ainsi, M1 semble posséder une organisation somatotopique générale, où les segments majeurs du corps sont arrangés selon une disposition médio-latérale (Penfield and Boldrey, 1937; Woolsey et al., 1952). Cependant, à l'intérieur même de l'un de ces segments majeurs, par exemple le bras, les représentations des différents muscles se chevauchent considérablement. Afin de transmettre les commandes motrices volontaires aux différentes parties du corps, M1 tire parti de ses nombreuses connexions avec la moelle épinière. Plusieurs de ces projections corticospinales ciblent les segments cervicaux de la moelle épinière qui contiennent les motoneurones contrôlant les muscles de la main (He et al., 1993). Bien que la majorité des neurones de M1 projettent vers les interneurones de la moelle épinière (Maier et al., 2002), il a été démontré que certains possèdent des connexions directes avec les motoneurones innervant les muscles de la main et ce, seulement chez les animaux ayant une grande dextérité manuelle (c.-à-d. certaines espèces de primates) (Bortoff and Strick, 1993; Maier et al., 2002). Ainsi, il a été suggéré que les connexions directes entre M1 et la moelle épinière, appelées connexions cortico-motoneuronales (CM), supportent le mouvement indépendant des doigts qui est beaucoup plus développé chez les espèces possédant ce type de connexions (Lemon, 1993, 2008).

Le rôle de M1 dans le contrôle moteur du membre supérieur a fait, et fait toujours, l'objet de plusieurs débats. Evarts a été le premier à enregistrer l'activité de neurones individuels au sein de M1 chez le macaque éveillé (Evarts, 1968). Cette étude a démontré que la décharge neuronale

au sein de M1 varie avec le niveau de contraction des muscles. Ainsi, les résultats de cette expérience suggérèrent que les neurones de M1 sont essentiellement des contrôleurs musculaires: plus l'activité neuronale est grande au sein de M1, plus le signal envoyé au muscle est grand et donc plus grand est le mouvement. Lors d'études subséquentes, il a toutefois été démontré que la décharge des neurones de M1 est plutôt associée à un certain nombre de paramètres externes tels que la direction et la vitesse du mouvement (Georgopoulos et al., 1982; Ashe and Georgopoulos, 1994). En définitive, il a été déterminé que les neurones de M1 codent probablement un amalgame de paramètres représentant autant les aspects cinétique (force, activité musculaire) que cinématique (direction, vitesse) du mouvement (Kakei et al., 1999). Globalement, la décharge neuronale au sein de M1 semble ainsi étroitement liée aux aspects élémentaires du mouvement plutôt qu'à des aspects plus abstraits tel que le contexte comportemental, qui sont traités d'avantage par les aires prémotrices. Conséquemment, à l'instar des aires prémotrices, les neurones de M1 sont plus actifs lors de l'exécution que lors de la préparation du mouvement (Riehle and Requin, 1989). Allant dans ce sens, les lésions de M1 ont des effets profonds sur la performance motrice (Travis, 1955; Rouiller et al., 1998) et peuvent entraîner une diminution de la précision et de la vitesse du mouvement, une faiblesse musculaire et une perte d'individuation du mouvement des doigts, soulignant son rôle crucial dans l'exécution des mouvements de la main.

2.2. Les aires prémotrices

Les aires prémotrices sont des aires frontales, situées rostralement à M1 (Figure 1.1). Elles sont définies comme étant des aires corticales ayant des projections directes vers M1 et la moelle épinière (Dum and Strick, 2002). Elles incluent deux aires situées sur la surface latérale du cortex: le cortex prémoteur ventral (PMv) et le cortex prémoteur dorsal (PMd) et plusieurs aires situées sur le mur médian du cortex comprenant l'aire motrice supplémentaire (SMA) et les aires motrices cingulaires (CMA). Étant donné l'accès difficile aux CMA dû à leur emplacement au sein du sulcus cingulaire, ces dernières n'ont pas été examinées lors de nos études et ne seront donc pas discutées dans cette thèse. De manière générale, les aires prémotrices sont associées à des processus corticaux plus complexes que M1. Notamment, elles semblent impliquées à la fois dans la préparation et l'exécution de mouvements volontaires alors que M1 semble principalement impliqué dans l'exécution de ces mouvements.

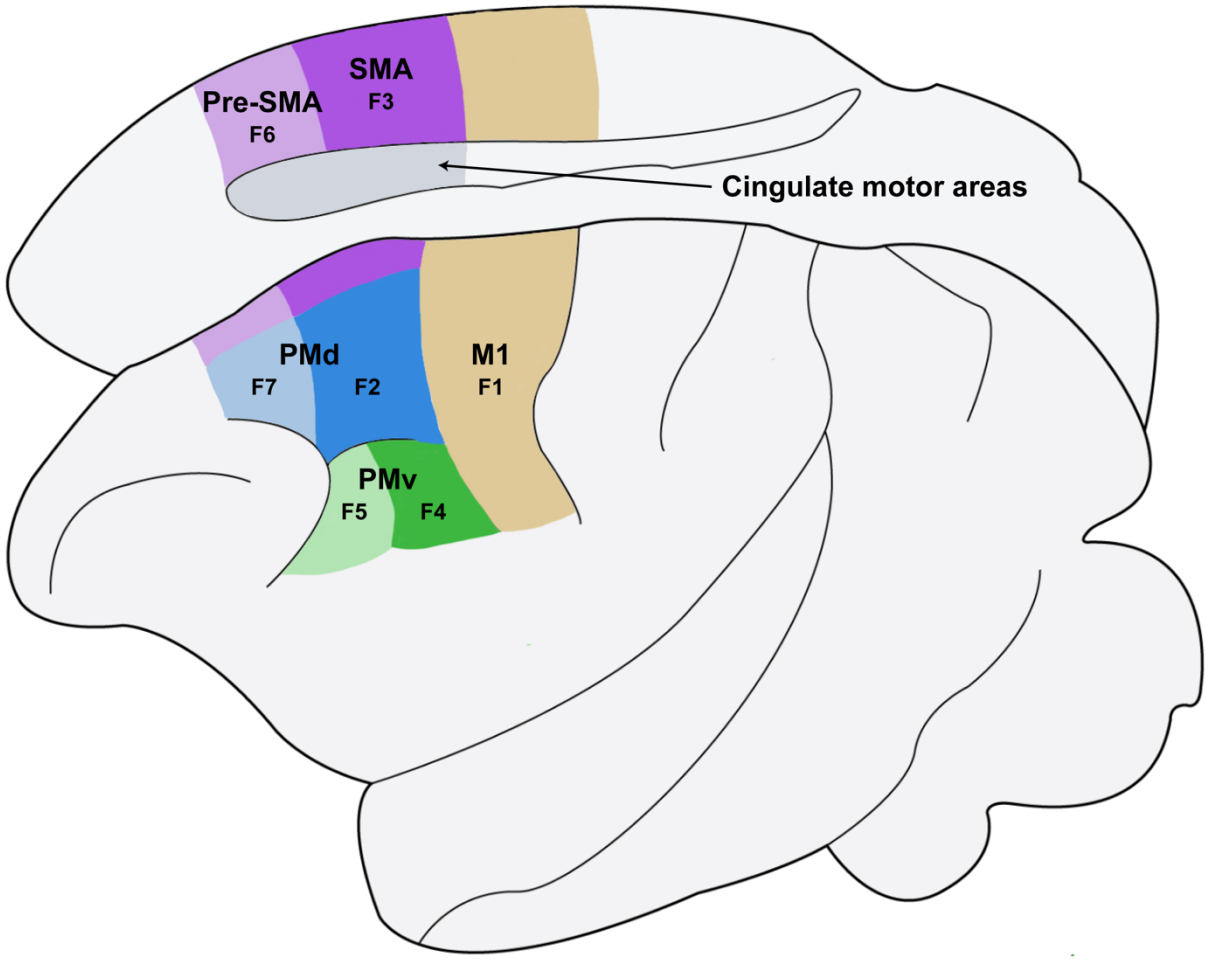

Figure 1.1 *Les aires corticales motrices.*

Schéma résumant la division des différentes aires motrices corticales sur la surface latérale (en bas) et sur la surface médiale (en haut) du cortex selon les nomenclatures les plus répandues dans la littérature. Sur la surface latérale du cortex, nous retrouvons M1 (F1), PMv qui est sous-divisé en deux territoires: F5 et F4 ainsi que PMd qui est également sous-divisé en deux territoires: F7 et F2. Sur la surface médiale du cortex, nous retrouvons Pré-SMA (F6), SMA (F3) ainsi que les CMA.

L'exécution d'une action orientée vers un but requiert la transformation d'informations visuelles et somesthésiques en commandes motrices appropriées. Par exemple, saisir une tasse de café nécessite de l'information quant à l'emplacement de la tasse et de la main dans l'espace, à l'emplacement de l'une par rapport à l'autre et aux caractéristiques physiques de la tasse. De plus, ce type d'action requiert d'organiser temporellement une séquence de mouvement en se basant sur des informations gardées en mémoire. Par exemple, boire une tasse de café nécessite de tendre la main vers la tasse, la saisir, la soulever et la transporter vers la bouche. Ces informations sensorielles et cognitives sont intégrées par les aires prémotrices afin de planifier des commandes motrices adaptées à différentes situations. De manière intéressante, chaque aire prémotrice semble jouer un rôle spécialisé dans la préparation et l'exécution des mouvements volontaires.

2.2.1. Le cortex prémoteur ventral (PMv)

Le cortex prémoteur ventral (PMv) est situé sur la surface latérale du cortex, latéralement au genu du sillon arqué. Des études de microstimulation chez le singe ont démontré que tout comme M1, PMv contient une représentation des mouvements du bras en plus d'une représentation des mouvements orofaciaux (Gentilucci et al., 1988; Rizzolatti et al., 1988; Hepp-Reymond et al., 1994; Preuss et al., 1996). L'intensité de stimulation nécessaire pour évoquer des mouvements dans PMv est toutefois plus élevée que celle utilisée dans M1 (>15µA pour PMv et aussi peu que 5µA pour M1) (Preuss et al., 1996; Dancause et al., 2008). En se basant sur des différences anatomiques et fonctionnelles, PMv a été subdivisé en deux territoires: PMv-caudal (PMv-c ou F4) et PMv-rostral (PMv-r ou F5) (Geyer et al., 2000) (Figure 1.1). D'après les mouvements évoqués par la microstimulation, le territoire cortical évoquant des mouvements proximaux du bras fait principalement partie de PMv-caudal (ou F4) alors que PMv-rostral (ou F5) évoque majoritairement des mouvements de la main. Contrairement aux autres aires prémotrices qui envoient des projections corticospinales vers tous les segments cervicaux de la moelle épinière, les neurones corticospinaux situés dans la représentation de la main de PMv projettent principalement vers les segments cervicaux supérieurs contenant les motoneurones innervant les muscles du cou (He et al., 1993; Galea and Darian-Smith, 1994; Borra et al., 2010; Morecraft et al., 2019). Puisque les motoneurones contrôlant les muscles distaux du bras sont situés au niveau des segments cervicaux inférieurs, il a donc été suggéré que les mouvements distaux produits par la stimulation

de PMv sont majoritairement évoqués à travers les connexions cortico-corticales reliant PMv et la représentation de la main de M1.

Les premières études électrophysiologiques étudiant la fonction de PMv l'ont caractérisé comme étant impliqué dans les mouvements sous guidage visuel car les neurones localisés dans F4 et F5 (mais surtout F4) déchargent lors de la préparation et l'exécution de mouvements guidés, entre autres, par la vision (Kurata and Tanji, 1986; Gentilucci et al., 1988; Rizzolatti et al., 1988). En accord avec cette notion, une grande proportion des neurones de PMv possède des champs récepteurs visuels qui encodent les caractéristiques des objets présents dans l'espace péripersonnel (Gentilucci et al., 1988; Rizzolatti et al., 1988; Murata et al., 1997). Du point de vue moteur, les neurones de PMv impliqués dans la production de mouvements distaux montrent une sélectivité pour différents types de configuration de la main. Par exemple, il a été démontré chez le macaque entrainé à une tâche d'atteinte et de préhension de différents objets que les neurones de PMv sont sélectifs pour trois types de préhension de base: la préhension de précision (opposition du pouce et de l'index), la préhension avec les doigts (opposition du pouce avec tous les autres doigts) et la préhension avec la main entière (flexion de tous les doigts autour de l'objet) (Rizzolatti et al., 1988). Il a été démontré que cette préférence est également présente lors de l'observation de ces mêmes objets, dans un contexte qui ne requiert aucun mouvement (Murata et al., 1997; Raos et al., 2006). Ainsi, il a été proposé que les neurones de PMv transforment les propriétés physiques des objets en actions motrices appropriées à la préhension de ces derniers. Appuyant ces résultats électrophysiologiques, des études chez le singe et l'humain ont rapportés des déficits précis à la suite d'une inactivation transitoire de PMv. Par exemple, il a été démontré chez des singes entrainés à une tâche d'atteinte et de préhension de différents objets, que l'inactivation de PMv via l'injection d'un agoniste du neurotransmetteur GABA génère des déficits importants au niveau de la configuration des doigts, engendrant une posture de la main inappropriée pour la taille et la forme de l'objet (Fogassi et al., 2001). Chez l'humain, la perturbation de l'activité neuronale de PMv via une stimulation magnétique transcrânienne répétitive (rTMS) entraine également des déficits dans le positionnement des doigts précédant la manipulation d'un objet (Davare et al., 2006). Finalement, des études d'imagerie chez l'humain ont montré qu'une augmentation du flux sanguin cérébral a lieu au sein de PMv lors de la préhension de précision et la manipulation d'un objet (Binkofski et al., 1999; Ehrsson et al., 2001; Kuhtz-Buschbeck et al., 2001).

En résumé, PMv joue un rôle crucial dans le contrôle des mouvements de la main chez le primate. Plus précisément, PMv semble transformer les caractéristiques physiques des objets en commandes motrices appropriées à leur préhension. Ceci permettrait à PMv d'élaborer des configurations précises de la main et des doigts en fonction de la tâche à accomplir.

2.2.2 Le cortex prémoteur dorsal (PMd)

Le cortex prémoteur dorsal (PMd) est situé sur la surface latérale du cortex, médialement au genu du sillon arqué. Tout comme PMv, des études de microstimulation chez le singe ont révélé la présence d'une représentation du bras au sein de PMd qui requiert une intensité de stimulation plus forte que dans M1 pour éliciter des mouvements (>20-30µA pour PMd et aussi peu que 5µA pour M1) (Preuss et al., 1996; Raos et al., 2003). Également à l'exemple de PMv, PMd a été subdivisé en deux sous-régions: PMd-caudal (PMd-c ou F2) et PMd-rostral (PMd-r ou F7) (Geyer et al., 2000) (Figure 1.1). La représentation de la main est située dans la section latérale de PMd-c, qui possède également une représentation de la jambe. PMv-r contient une représentation du cou, du tronc, du visage et des yeux (Preuss et al., 1996). Contrairement à PMv, les neurones corticospinaux de la représentation de la main de PMd projettent vers les segments cervicaux inférieurs qui contiennent les motoneurones innervant les muscles distaux du bras et semble donc être en mesure d'influencer plus directement les mouvements de la main (He et al., 1993). Toutefois, PMd envoient aussi des projections vers la représentation de la main de M1 et peut donc influencer le mouvement via ces connexions cortico-corticales (Marconi et al., 2003; Hamadjida et al., 2016).

Wise et collègues ont été parmi les premiers à étudier les propriétés fonctionnelles des neurones de PMd. Ils ont montré chez des singes entrainés à exécuter des mouvements d'atteinte basés sur des instructions visuo-spatiales que les neurones de PMd sont particulièrement actifs lors de la préparation, mais déchargent aussi lors de l'exécution du mouvement (Weinrich and Wise, 1982; Kurata and Wise, 1988; Kurata and Hoffman, 1994). Pendant la planification du mouvement d'atteinte, PMd semble intégrer l'information concernant la position de la cible à atteindre et le bras à utiliser afin de spécifier l'action adéquate à accomplir (Hoshi and Tanji, 2000, 2004). Les neurones de PMd codent également la position de la cible par rapport à la position de la main et de l'œil lors de la planification du mouvement d'atteinte (Pesaran et al., 2006). Finalement, de nombreuses études ont révélé que l'activité neuronale de PMd est corrélée aux paramètres du mouvement tels que la direction, l'amplitude et la trajectoire (Hocherman and Wise, 1990; Fu et

al., 1993; Crammond and Kalaska, 2000). Ainsi, il est proposé qu'une des fonctions principales de PMd est de traiter les informations visuo-spatiales concernant la cible et l'effecteur afin de préparer et de guider la position du bras pendant la phase de transport vers la cible (Rizzolatti et al., 1988).

Bien que plusieurs mouvements volontaires soient dirigés vers des cibles ou objets, ils peuvent également être guidés par des règles arbitraires (ex. démarrer au feu vert et arrêter au feu rouge). Une autre fonction de PMd semble être de sélectionner des mouvements qui sont basés sur de telles associations visuo-motrices. Par exemple, l'inactivation temporaire de PMd chez le macaque est caractérisée par des erreurs de direction lors de la sélection de mouvements basés sur des instructions visuelles arbitraires (ex. extension du poignet en réaction à une lumière verte et flexion du poignet en réaction à une lumière rouge) (Halsband and Passingham, 1985; Petrides, 1986; Kurata and Hoffman, 1994). De manière similaire, l'inactivation de PMd chez l'humain via le rTMS altère la capacité à sélectionner une réponse motrice adéquate lors d'une tâche impliquant des associations visuomotrices arbitraires (Chouinard et al., 2005). Ces résultats suggèrent que PMd a un rôle majeur à jouer en ce qui concerne les mouvements dont la sélection dépend de règles arbitraires incorporées dans différents contextes environnementaux.

Dans la majorité des travaux cités plus haut, seuls les paramètres codés par les neurones de PMd lors les mouvements d'atteinte étaient étudiés. Toutefois, lorsqu'une étude a examiné la décharge neuronale associée aux mouvements de préhension dans PMd, il a été démontré que tout comme PMv, PMd possèdent des neurones qui sont sélectifs pour le type de préhension utilisé pour saisir différents objets lors de la préparation et l'exécution du mouvement de saisie (Raos et al., 2004). S'appuyant sur l'existence de connexions anatomiques entre PMd et PMv, ces chercheurs proposent que PMv fourni une représentation motrice de l'objet à saisir à PMd qui combinerait cette représentation aux informations visuo-spatiales du contexte environnemental afin de continuellement mettre à jour la configuration et l'orientation de la main lors de l'approche vers l'objet à saisir (Raos et al., 2004).

En résumé, PMd joue un rôle crucial dans le contrôle des mouvements d'atteinte et de saisie chez le primate. Plus précisément, PMd semble préparer et guider en temps réel ces mouvements en se basant sur des informations visuo-spatiales. En outre, PMd semble être particulièrement impliqué dans la sélection de mouvements reposant sur des associations visuomotrices arbitraires.

2.2.3. L'aire supplémentaire motrice (SMA)

L'aire supplémentaire motrice (SMA) est située sur la surface médiale du cortex, rostralement à la représentation de la jambe de M1. Tout comme PMv et PMd, des études utilisant la ICMS ont démontré que SMA possède une représentation du bras (Gould et al., 1986; Mitz and Wise, 1987; Luppino et al., 1991). Basé sur des évidences anatomiques et fonctionnelles, SMA a été subdivisé en deux zones: une zone caudale (SMA ou F3) et une zone rostrale (pré-SMA ou F6) (Luppino et al., 1993; Geyer et al., 2000) (Figure 1.1). SMA possède une représentation complète du corps avec la représentation du membre postérieur, du membre antérieur et de la tête retrouvés dans un ordre caudo-rostral (Gould et al., 1986; Mitz and Wise, 1987; Luppino et al., 1991). Les mouvements y sont évoqués via des intensités de stimulation relativement plus hautes que dans M1 (Luppino et al., 1991). À l'opposé, seule une représentation du bras est présente au sein de pré-SMA, qui requiert une intensité de stimulation plus élevée que SMA pour évoquer des mouvements. De plus, ceux-ci sont généralement lents et toniques (Luppino et al., 1991; Matsuzaka et al., 1992). Tout comme PMd, les neurones de SMA projettent vers les segments cervicaux inférieurs de la moelle épinière (Dum and Strick, 1991; He et al., 1993; Maier et al., 2002). Maier et collègues (2002) ont même démontré que SMA possède des connexions directes (CM) avec des motoneurones dans la moelle épinière innervant les muscles de la main. Cependant, celles-ci sont moins nombreuses et puissantes que celle provenant de M1. Finalement, tout comme PMv et PMd, SMA possède des connexions substantielles avec la représentation de la main de M1 (Dum and Strick, 2005; Hamadjida et al., 2016). À l'opposé, pré-SMA ne possèdent pas de projections vers la moelle épinière et peu de connexions cortico-corticales avec M1 (Dum and Strick, 1991; Luppino et al., 1993; Dum and Strick, 1996). Ainsi, il est suggéré qu'SMA est une région prémotrice alors que pré-SMA est plutôt une région préfrontale. Dans le contexte de cette thèse, nous nous pencherons uniquement sur SMA compte tenu de sa fonction motrice.

Initialement, des études chez le macaque ont démontré que l'activité neuronale au sein de SMA est modulée avant et pendant des mouvements simples de la main (Tanji and Kurata, 1979, 1982). Chez les sujets humains, une augmentation du flux sanguin cérébral a également été observée lors de la préparation et l'exécution de mouvements qui ne sont pas organisés de manière complexe (Colebatch et al., 1991; Grafton et al., 1993). Ces données semblent indiquer que SMA est impliqué dans le contrôle des mouvements simples. Toutefois, d'autres évidences portent à

croire que l'activité de SMA n'est pas principalement associée à de tels mouvements. En fait, l'augmentation du flux sanguin cérébral lors de l'exécution de mouvements simples est beaucoup moins importante dans SMA que dans M1 (Colebatch et al., 1991; Grafton et al., 1993). De plus, à la suite d'une lésion ou d'une inactivation de SMA, l'absence de déficits observé lors de l'exécution de mouvements simples suggère que le rôle de SMA est subsidiaire à celui de M1 pour ce type de mouvement (Brinkman, 1984; Tanji et al., 1985).

En revanche, des études chez l'humain et le singe ont révélé que SMA est étroitement impliqué dans le contrôle de mouvements plus complexes. Des études d'imagerie chez l'humain ont montré une augmentation considérable du flux sanguin cérébral au sein de SMA lors de l'exécution de séquences de mouvements complexes (Orgogozo and Larsen, 1979; Roland et al., 1980b). De plus, des études chez le macaque ont démontré que les neurones de SMA sont spécifiquement impliqués dans le séquençage temporel de plusieurs mouvements (Mushiake et al., 1990; Halsband et al., 1994; Tanji and Shima, 1994). En lien avec ces résultats, la lésion ou l'inactivation de SMA chez le macaque et l'humain affectent profondément le séquençage temporel des mouvements de la main et ce, particulièrement lorsqu'ils sont basés sur la mémoire (Brinkman, 1984; Dick et al., 1986; Halsband et al., 1993; Shima and Tanji, 1998). Ces déficits se manifestent peu importe la main utilisée, qu'elle soit ipsilatérale ou contralatérale à la lésion. Un autre effet observé à la suite d'une lésion de SMA est l'incapacité, à long terme, d'effectuer des mouvements cordonnés entre les deux mains (Laplane et al., 1977; Brinkman, 1984; Halsband et al., 1993). En accord avec ces données, des études électrophysiologiques ont démontré qu'un nombre important de neurones décharge spécifiquement lors de mouvements bimanuels au sein de SMA (Tanji et al., 1987, 1988; Kermadi et al., 1998).

En résumé, bien que les neurones de SMA soient actifs lors de tâches simples, cette aire prémotrice semble principalement impliquée dans la préparation et l'exécution de mouvements complexes qui nécessitent un séquençage temporel de plusieurs mouvements et/ou une coordination bimanuelle.

3. Connexions anatomiques entre les aires prémotrices et M1

Les aires prémotrices et M1 situés dans le même hémisphère cérébral possèdent de nombreuses interconnexions anatomiques à travers lesquelles ces régions peuvent s'influencer pour la production du mouvement. Les aires prémotrices d'un hémisphère cérébral et M1 de l'hémisphère opposé sont également interconnectées anatomiquement, principalement via le corps calleux, leur permettant aussi d'interagir pour contrôler le mouvement. Finalement, les aires prémotrices envoient des projections vers des structures sous-corticales qui sont également innervées par les projections de M1, offrant d'autres sites d'interactions potentiels. Dans la section suivante, nous examinerons les connexions anatomiques qui relient les aires prémotrices et M1 avec une emphase sur les connexions qui sous-tendent les mouvements de la main.

3.1. Connexions intrahémisphériques entre les aires prémotrices et M1

À travers des études de traçage neuroanatomique chez le primate non-humain, il a été démontré que les deux sources majeures d'afférences du lobe frontal vers la représentation de la main de M1 proviennent de PMd et de PMv (Dum and Strick, 2005; Dancause et al., 2006b). Bien que la troisième plus grande source de projections tire son origine de SMA, le nombre de neurones de SMA qui contribuent à ces projections est inférieur de moitié à ceux de PMd ou de PMv. En fait, SMA semble envoyer plus de projections vers la représentation de la main de PMd et PMv que vers celle de M1 (Dum and Strick, 2005).

De manière importante, la représentation de la main de PMd et de PMv reçoit également de nombreuses projections issues de M1. Ainsi, il a été proposé que PMv, PMd et M1 forment un réseau cortical densément interconnecté qui est fortement impliqué dans la production et le contrôle des mouvements de la main (Dum and Strick, 2005). Plus récemment, des études dans notre laboratoire chez le singe capucin ont démontré que chaque aire prémotrice est préférentiellement interconnectée avec une sous-région spécifique de M1 (Dea et al., 2016; Hamadjida et al., 2016). Par exemple, PMv est préférentiellement interconnecté avec la région rostro-latérale, PMd avec la région rostro-médiale et SMA avec la région caudo-médiale (Figure 1.1). Ces données supportent l'idée selon laquelle M1 est composé des plusieurs modules, qui sont ciblés par différentes aires prémotrices afin de soutenir des fonctions spécialisées.

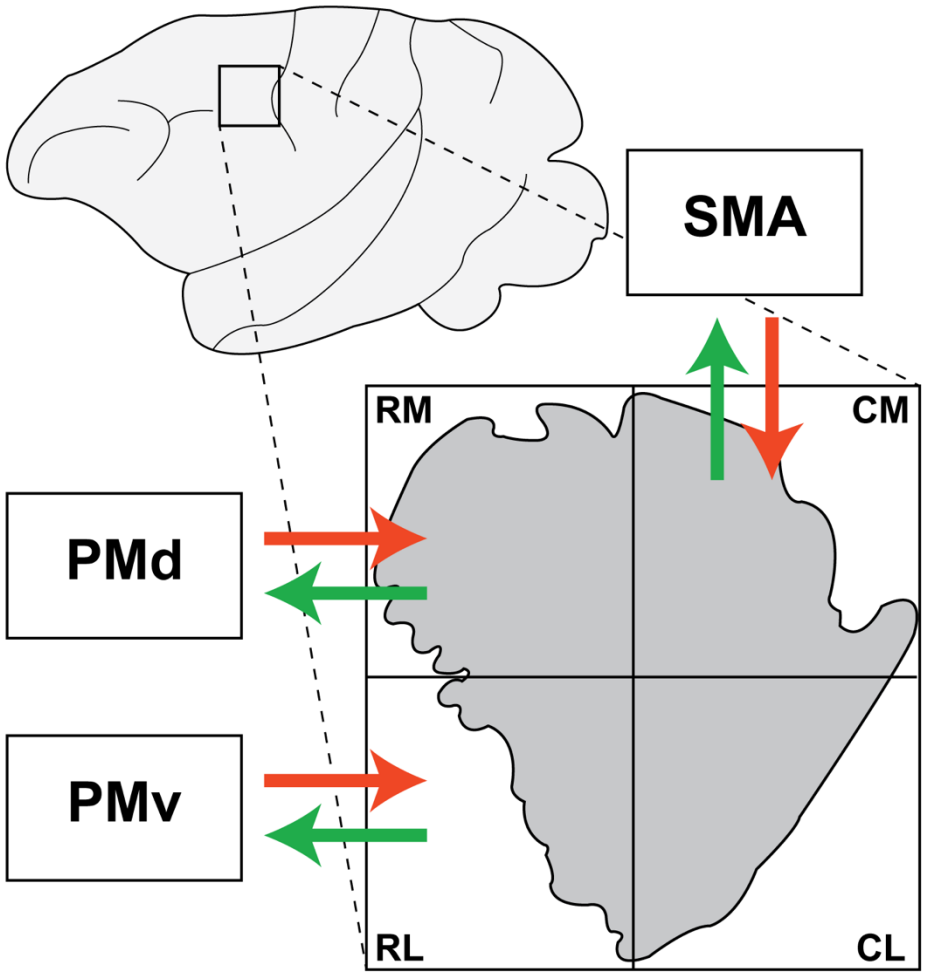

Figure 1.2 *Afférences et efférences intrahémisphériques entre les aires prémotrices et M1.*

En haut, vue de la surface du cortex où le rectangle indique la localisation de la représentation de la main de M1 qui est illustrée en détail plus bas. En bas, ségrégation des afférences (rouge) et efférences (vert) intrahémisphériques entre la représentation de la main des différentes aires prémotrices et des différentes sous-régions de la représentation de la main de M1 (gris). RM: rostro-médial; RL: rostro-latéral; CM: caudo-médial; CL: caudo-latéral. Modifiée de Hamadjida et al. (2016).

3.2. Connexions interhémisphériques entre les aires prémotrices et M1

En plus du réseau de connexions intrahémisphériques, un réseau interhémisphérique relie les aires prémotrices et M1 des deux hémisphères à travers le corps calleux. Plusieurs études neuroanatomiques ont démontré que chaque aire prémotrice est principalement interconnectée avec son homologue de l'hémisphère opposé (Rouiller et al., 1994; Marconi et al., 2003; Boussaoud et al., 2005; Dancause et al., 2007). Par exemple, environ 45% des projections interhémisphériques de la représentation de la main de PMv ciblent le PMv de l'hémisphère opposé (Dancause et al., 2007). En comparaison, environ 11% de ces projections ciblent M1, et ce, principalement dans la portion rostrale de la représentation de la main. Ainsi, bien qu'il existe des connexions anatomiques reliant les aires prémotrices et la représentation de la main de M1 de l'hémisphère opposé, ces dernières semblent beaucoup moins nombreuses que celles qui sont situées dans le même hémisphère (Muakkassa and Strick, 1979; Dancause et al., 2007). Il est important de préciser que les connexions interhémisphériques entre les aires prémotrices et M1 peuvent également se produire indirectement à travers les connexions reliant les aires prémotrices des deux hémisphères (ex. PMv droit → PMv gauche → M1 gauche). Les nombreuses connexions associant les représentations de la main des aires prémotrices des deux hémisphères pourraient être utilisées pour échanger de l'information lors de la préparation du mouvement, par exemple lorsque ceux-ci nécessitent la collaboration des deux mains (Rouiller et al., 1994; Dancause et al., 2007).

3.3. Connexions sous-corticales des aires prémotrices et de M1

Outre les connexions cortico-corticales reliant les aires prémotrices et M1, ces aires motrices corticales possèdent des projections en commun vers plusieurs structures sous-corticales telles que le noyau rouge, la formation réticulée et la moelle épinière. Ces projections sous-corticales convergentes pourraient, tout comme les connexions cortico-corticales décrites plus haut, offrir un substrat anatomique permettant aux aires prémotrices d'interagir avec les efférences de M1.

3.3.1. Connexions avec le noyau rouge

Le noyau rouge situé au niveau du mésencéphale est la source de la voie rubrospinale (RuST), une voie descendante motrice qui influence les mouvements de la main à travers ses projections vers la moelle épinière. Les projections de la RuST ciblent particulièrement les segments spinaux

innervant les muscles distaux et semblent terminées directement sur les motoneurones impliqués dans les mouvements de la main (Holstege et al., 1988; Ralston et al., 1988). Ces évidences anatomiques sont supportées par des études électrophysiologiques chez le singe démontrant que le noyau rouge est fortement impliqué lors de différents mouvements de la main (Gibson et al., 1985; Mewes and Cheney, 1991; Belhaj-Saif et al., 1998). Puisque les aires prémotrices et M1 projettent vers le noyau rouge (Kuypers and Lawrence, 1967; Monakow et al., 1979) et que ce dernier semble être en mesure d'influencer la production des mouvements de la main, un site d'interaction en son sein où les aires prémotrices pourraient moduler les efférences de M1 vers les muscles de la main semble concevable.

3.3.2. Connexions avec la formation réticulée

La formation réticulée pontomedullaire (PMRF) située dans le tronc cérébral est la source de la voie réticulospinale (RST), une voie descendante motrice bilatérale qui joue un rôle bien établi dans les mouvements du bras via ses projections vers la moelle épinière (Sakai et al., 2009). De manière intéressante, des études chez le singe ont démontré que les neurones réticulospinaux peuvent activer les interneurones et les motoneurones de la moelle épinière impliqués dans le contrôle des muscles de la main et peut moduler leur activité lors du mouvement des doigts (Riddle et al., 2009; Riddle and Baker, 2010; Soteropoulos et al., 2012). Ainsi, la RST semble exercer une certaine influence sur les mouvements de la main. Dans cette optique, les études anatomiques démontrant que PMv, PMd, SMA et M1 projettent tous vers la PMRF sont importants à considérer (Kuypers and Lawrence, 1967; Monakow et al., 1979; Keizer and Kuypers, 1989; Fregosi et al., 2017). En effet, il est possible que la PMRF offre un autre site d'interaction au sein duquel les aires prémotrices peuvent moduler les efférences motrices de M1 vers les muscles de la main.

3.3.3. Connexions avec la moelle épinière

Tel que décrit précédemment, les aires prémotrices et M1 contribuent à la voie corticospinale (CST) reliant directement le cortex à la moelle épinière (Dum and Strick, 1991; He et al., 1993, 1995). Tout comme M1, les projections provenant des aires prémotrices terminent principalement dans la zone intermédiaire de la moelle épinière où se trouvent les interneurones et non sur les motoneurones elles-mêmes (Maier et al., 2002; Morecraft, 2019). Toutefois, des différences

nettes se dessinent entre les aires prémotrices et M1 lorsque l'on considère les projections cortico-motoneuronales (CM). En l'occurrence, une étude comparant les connexions CM provenant de SMA et de M1 a démontré que celles provenant de SMA était beaucoup moins denses et puissantes que celles provenant de M1 (Maier et al., 2002). Malgré cet impact moins important des projections CM issues de SMA, la même étude a mis en évidence la présence de motoneurones recevant des afférences convergentes de SMA et de M1, illustrant le potentiel de SMA à moduler les efférences de M1 au niveau spinal de manière directe. En plus de cette modulation directe sur les motoneurones, les projections corticospinales d'une aire prémotrice peuvent également moduler les efférences de M1 en projetant sur les mêmes interneurones que ce dernier ou plus indirectement en modulant l'excitabilité générale de la circuiterie spinale reliée aux mouvements de la main. Toutefois, ceci nécessite que l'aire prémotrice en question innerve les segments spinaux impliqués dans de tels mouvements (C6-T1). Ceci est le cas pour SMA et PMd, mais est moins clair pour PMv (He et al., 1993, 1995; Borra et al., 2010; Morecraft et al., 2019). De manière étonnante, les projections corticospinales de PMv semblent principalement innerver les segments spinaux supérieurs (C2-C4) qui ne contrôlent pas les muscles de la main (He et al., 1993; Borra et al., 2010; Morecraft et al., 2019). Cependant, il a été proposé que les projections corticospinales de PMv terminant sur les neurones propriospinaux des segments C3-C4, qui eux, innervent les segments impliqués dans les mouvements de la main, pourrait permettre à PMv d'influencer indirectement ces derniers (Borra et al., 2010; Kinoshita et al., 2012). En somme, les projections corticospinales des différentes aires prémotrices, en particulier celles issues SMA et PMd, les placent dans une position stratégique pour influencer, au niveau spinal, les efférences de M1 vers les muscles de la main.

4. Interactions fonctionnelles entre les aires prémotrices et M1

Une façon dont les aires prémotrices peuvent participer à la production des mouvements de la main est de moduler les efférences motrices de M1 à travers les connexions anatomiques décrites dans la section précédente. Plusieurs études chez l'humain et le singe ont examiné les effets modulateurs des aires prémotrices sur les efférences de M1 vers les muscles de la main en utilisant des protocoles de stimulations pairées via la stimulation magnétique transcrânnienne (TMS) ou la ICMS. Au cours de ces protocoles, des stimulations simples sont appliquées dans une aire prémotrice et dans M1 alors que l'activité des muscles du côté contralatéral à M1 est quantifiée. Différents types de protocoles utilisant diverses intensités de stimulation peuvent être employés. Dans le contexte de cette thèse, nous nous intéresserons principalement au des protocoles où une stimulation appliquée dans l'aire prémotrice, dite conditionnante (C), est sous le seuil évoquant des effets moteurs alors que celle de la stimulation appliquée dans M1, dite test (T), excède ce seuil. Au sein de chaque protocole, une stimulation peut être envoyée seulement dans l'aire prémotrice (C-only), seulement dans M1 (T-only) ou peut être envoyée dans l'aire prémotrice suivie, à différents intervalles interstimulus (ISIs) de quelques millisecondes (ms), d'une stimulation dans M1 (C+T ou stimulations pairées). Afin de déterminer si l'aire prémotrice module les efférences de M1, les réponses musculaires induites par les stimulations C-only et T-only sont additionnées (prédicteur) puis l'amplitude de cette réponse est comparée à celle de la réponse induite lors des stimulations pairées aux différents ISIs. Si la réponse musculaire induite par le prédicteur est égale à celle induite lors des stimulations pairées, l'aire prémotrice est considérée comme n'ayant pas eu d'effets modulateurs sur les efférences de M1. Par contre, si la réponse musculaire induite par le prédicteur est significativement plus petite ou plus grande que celle induite par les stimulations pairées, il est estimé que l'aire prémotrice a eu un effet facilitateur ou inhibiteur sur les efférences de M1, respectivement. Ainsi, cette technique permet de caractériser les interactions fonctionnelles intra et interhémisphériques reliant les aires prémotrices et M1. Différents patrons de modulation provenant des diverses aires prémotrices pourraient fournir un substrat à travers lequel celles-ci assument leurs rôles uniques dans la préparation et la production des mouvements de la main.

4.1. Interactions fonctionnelles entre PMv et M1

Plusieurs études chez le macaque ont investigué les effets modulateurs du PMv ipsilatéral (iPMv) à M1 sur les efférences de ce dernier vers les muscles de la main à l'aide de protocoles de stimulation pairées (Cerri et al., 2003; Shimazu et al., 2004; Prabhu et al., 2009). Chez le macaque sous sédation, il a été démontré que bien qu'une stimulation de basse intensité dans iPMv ne produise peu ou pas d'effets sur l'activité des muscles de la main, lorsqu'elle est pairée à une stimulation dans M1, elle peut faciliter les efférences de M1 vers ces mêmes muscles à divers ISIs (1-15 ms) (Cerri et al., 2003). Par ailleurs, une étude chez le macaque exécutant des mouvements d'atteinte et de saisie vers des objets a montré que iPMv semble faciliter les efférences de M1 vers les muscles de la main avec des ISIs courts (0-1 ms) et les inhiber avec des ISIs plus longs (5-6 ms) (Prabhu et al., 2009). La facilitation n'était présente que dans certains muscles et lors de l'atteinte et la saisie d'un sous-ensemble d'objets. Ainsi, les effets facilitateurs de iPMv sur les efférences de M1 semble spécifique au type de préhension utilisé. Finalement, chez le macaque anesthésié, une stimulation de iPMv entraîne une facilitation des potentiels postsynaptiques excitateurs des motoneurones de la main induis par une stimulation de M1 (Shimazu et al., 2004).

Chez l'humain, des études utilisant des protocoles de stimulations pairées ont également rapporté que iPMv peut avoir des effets inhibiteurs et facilitateurs sur les efférences de M1 (Civardi et al., 2001; Munchau et al., 2002; Davare et al., 2008; Davare et al., 2009). Notamment, Davare et collègues (2008) ont démontrés que la nature des interactions entre iPMv et M1 dépend de l'état du système et du type de mouvement qui est exécuté. Lors du repos, les interactions sont majoritairement inhibitrices alors que pendant une préhension de force l'inhibition disparait et pendant une préhension de précision un effet facilitateur apparait. Dans une autre étude, Davare et collègues (2009) ont également démontré que lors de la préparation d'un mouvement de préhension d'objets, une stimulation envoyée dans iPMv 6 ou 8ms avant une stimulation dans M1 peut grandement faciliter l'activité des muscles spécifiquement utilisés lors de l'exécution du mouvement à venir.

Bien que les effets de iPMv aient été considérablement étudiés, ceux provenant de PMv situé dans l'hémisphère opposé à M1 (PMv contralatéral, cPMv) l'ont été beaucoup moins. Pourtant, chez le macaque, de nombreux neurones au sein de PMv déchargent lors de mouvements

exécutés avec l'une ou l'autre main (Rizzolatti et al., 1988; Tanji et al., 1988). En outre, chez l'humain, le mouvement séquentiel des doigts est associé à une augmentation de l'activité hémodynamique dans le PMv ipsilatéral à la main en mouvement (Hanakawa et al., 2005). Ces résultats suggèrent que cPMv peut influencer les mouvements de la main en modulant les efférences de M1 à travers les connexions interhémisphériques qui les relient. En effet, la seule étude ayant étudié les effets modulateurs de cPMv sur M1 a démontré que la nature de ceux-ci dépendait du contexte comportemental (Buch et al., 2010). Lors de la préparation et l'exécution d'un mouvement normal, cPMv avait un effet facilitateur sur les efférences de M1 alors que lorsque l'action initiale devait être supprimée et une nouvelle sélectionnée, cPMv devenait inhibiteur. À notre connaissance toutefois, aucune étude n'a encore directement comparé les effets modulateurs de iPMv et cPMv. Ceci est cependant nécessaire à examiner afin de mieux comprendre la contribution de chacune de ces régions corticales à la production des mouvements de la main. Par conséquent, nous avons examiné et comparé les effets modulateurs de iPMv et cPMv sur les efférences motrices de M1 vers les muscles de la main chez le singe capucin lors d'une étude présentée dans le **Chapitre 2**.

4.2. Interactions fonctionnelles entre PMd et M1

De nombreuses études de stimulations pairées se sont également penchées sur les effets modulateurs de PMd sur les efférences de M1 vers les muscles de la main. En utilisant le TMS chez l'humain, il a été démontré que le PMd ipsilatéral (iPMd) peut induire des effets facilitateurs ou inhibiteurs sur les efférences de M1 en fonction de l'ISI, de l'intensité du stimulus conditionnant ainsi que l'état du système (Civardi et al., 2001; Koch et al., 2007; Groppa et al., 2012; Vesia et al., 2018). Les effets modulateurs de iPMd sur M1 semblent être particulièrement prononcés lorsque les deux stimulations sont séparées par 6 ou 8ms. Au repos, les effets de iPMd sur les efférences de M1 sont inhibiteurs lorsque l'intensité du stimulus conditionnant est faible et facilitateurs lorsque l'intensité est augmentée (Civardi et al., 2001; Koch et al., 2007). Lors de la sélection ou la préparation d'un mouvement de la main contralatérale à M1, les effets de iPMd sont strictement facilitateurs (Groppa et al., 2012; Vesia et al., 2018). Toutefois, lorsque la main ipsilatérale à M1 est sélectionnée, iPMd inhibe les efférences de M1 (Groppa et al., 2012). De manière intéressante, il a été démontré que iPMd facilite les efférences de M1 lors de la préparation d'un mouvement de saisie vers un objet et ce, spécifiquement vers les muscles de la main

nécessaires à l'exécution du mouvement à venir (Vesia et al., 2018). Ceci suggère que, tout comme iPMv, les interactions fonctionnelles entre iPMd et M1 sont sélectivement modulées pendant la préparation des mouvements de saisie.

En ce qui concerne le PMd contalatéral à M1 (cPMd), des études de TMS chez l'humain ont démontré que ce dernier peut également faciliter (Baumer et al., 2006) ou inhiber (Mochizuki et al., 2004) les efférences de M1 vers les muscles de la main lors du repos dépendamment de l'intensité du stimulus conditionnant. Toutefois, à l'opposé de iPMd, les effets modulateurs de cPMd sur les efférences de M1 sont facilitateurs lorsque l'intensité du stimulus conditionnant est faible et inhibiteurs lorsqu'elle est plus forte. Les effets modulateurs provenant de cPMd sont principalement accentués lorsque des ISIs de 8 ou 10ms sont utilisés. Tout comme iPMd, cPMd induit des effets facilitateurs ou inhibiteurs sur les efférences de M1 au cours de la sélection du mouvement dépendamment de la main qui sera utilisée (Koch et al., 2006; O'Shea et al., 2007). Lorsque la main contralatérale à M1 est sélectionnée, cPMd a des effets facilitateurs sur les efférences de M1 alors que la situation opposée survient lorsque la main ipsilatérale à M1 est sélectionnée (Koch et al., 2006; O'Shea et al., 2007). En se basant sur ces résultats, il a été proposé qu'un des rôles de PMd (ipsi et contralatéral) est de faciliter l'exécution des mouvements sélectionnés et d'inhiber l'exécution de mouvements préparés mais non-sélectionnés. Puisque M1 est spécifiquement concerné par les mouvements de la main contralatérale, lorsqu'un mouvement de cette main est sélectionné, PMd faciliterait les efférences de M1 afin de favoriser l'exécution de ce mouvement. Au contraire, lorsqu'un mouvement de la main ipsilatérale à M1 est sélectionné, PMd inhiberait les efférences de M1 afin d'éviter de causer des mouvements indésirables du côté contralatéral à M1.

Bien que les effets modulateurs de PMd aient été considérablement étudiés, une question qui demeure inexplorée est comment ceux-ci diffèrent de ceux provenant de PMv lorsqu'étudiés dans les mêmes sujets. Différents patrons de modulation émanant de ces deux aires prémotrices pourraient fournir un substrat à travers lequel ces dernières pourraient assumer leurs rôles uniques dans la préparation et la production des mouvements de la main. Par conséquent, nous avons directement comparé les effets modulateurs provenant de PMd et de PMv dans les mêmes singes capucins lors d'une expérience présentée dans le **Chapitre 3**.

4.3. Interactions fonctionnelles entre SMA et M1

Contrairement à PMd et PMv, peu d'études ont étudiés les effets modulateurs de SMA sur les efférences de M1 (Oliveri et al., 2003; Arai et al., 2011; Fiori et al., 2017). Ces études de stimulations pairées chez l'humain via le TMS ont montré que SMA peut faciliter et inhiber les efférences de M1 vers les muscles de la main dépendamment des ISIs utilisés entre les deux stimulations. Cependant, puisque SMA est situé le long du mur médian du cerveau, il est techniquement difficile d'isoler les stimulations à l'intérieur du SMA ipsilatéral ou contralatéral (iSMA et cSMA) à M1 avec des techniques non-invasives tel que le TMS (Fiori et al., 2017). Ainsi, le profil de modulation spécifique à iSMA et cSMA demeure peu compris. De plus, dû aux divers protocoles expérimentaux utilisés dans les études de stimulations pairées chez l'humain, nous avons une compréhension incomplète des différences existant entre les effets modulateurs provenant de SMA, PMv et PMd. Par conséquent, nous avons examiné à l'aide de techniques invasives les effets modulateurs spécifiques de iSMA et cSMA sur les efférences de M1 vers les muscles de la main chez le singe capucin et les avons comparés à ceux induis par PMv et PMd dans les mêmes animaux lors d'une étude présentée dans le **Chapitre 4**.

5. Question de recherche, hypothèse générale et objectifs spécifiques

Cette thèse aborde la **question générale** de quels sont les effets modulateurs spécifiques induits par différentes aires prémotrices sur les efférences de M1 vers les muscles de la main chez le singe capucin. La thèse est divisée en trois chapitres qui relatent les résultats de trois études partageant l'objectif commun d'étudier les interactions fonctionnelles ayant lieu au sein du réseau moteur cortical. Ces travaux viennent étayer les études précédentes réalisées chez l'humain qui ont étudié ces interactions via des techniques non-invasives. L'originalité du travail repose sur une combinaison de techniques invasives permettant d'étudier de manière très détaillée les interactions entre les aires prémotrices et M1. Les chapitres répondent à l'**hypothèse générale** selon laquelle chaque aire prémotrice induit des effets modulateurs uniques sur les efférences motrices de M1 vers les muscles de la main.

L'**objectif spécifique du Chapitre 2** est de déterminer si le cortex prémoteur ventral (PMv) ipsilatéral et contralatéral à M1 engendrent des effets modulateurs similaires ou différents sur les efférences de M1.

L'**objectif spécifique du Chapitre 3** est de déterminer si le cortex prémoteur dorsal (PMd) ipisilatéral et contralatéral à M1 engendrent des effets modulateurs similaires ou différents sur les efférences de M1, puis de comparer ces effets à ceux de PMv décrits dans le Chapitre 1.

L'**objectif spécifique du Chapitre 4** est de déterminer si l'aire motrice supplémentaire (SMA) ipsilatérale et contralatérale à M1 engendrent des effets modulateurs similaires ou différents sur les efférences de M1, puis de comparer ces effets à ceux de PMv et de PMd décrits dans le Chapitre 2 et 3.

Chapitre 2 - Modulatory effects of the ipsi and contralateral ventral premotor cortex (PMv) on the primary motor cortex (M1) outputs to intrinsic hand and forearm muscles in *Cebus apella*

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Abstract

The ventral premotor cortex (PMv) is a key node in the neural network involved in grasping. One way PMv can carry out this function is by modulating the outputs of the primary motor cortex (M1) to intrinsic hand and forearm muscles. As many PMv neurons discharge when grasping with either arm, both PMv within the same hemisphere (ipsilateral; iPMv) and in the opposite hemisphere (contralateral; cPMv) could modulate M1 outputs. Our objective was to compare modulatory effects of iPMv and cPMv on M1 outputs to intrinsic hand and forearm muscles. We used paired-pulse protocols with intracortical microstimulations in capuchin monkeys. A conditioning stimulus was applied in either iPMv or cPMv simultaneously or prior to a test stimulus in M1 and the effects quantified in electromyographic signals. Modulatory effects from iPMv were predominantly facilitatory, and facilitation was much more common and powerful on intrinsic hand than forearm muscles. In contrast, while the conditioning of cPMv could elicit facilitatory effects, in particular to intrinsic hand muscles, it was much more likely to inhibit M1 outputs. These data show that iPMv and cPMv have very different modulatory effects on the outputs of M1 to intrinsic hand and forearm muscles.

Introduction

The ventral premotor cortex (PMv) is an area of the frontal lobe with a large representation from which hand movements can be evoked (Gentilucci et al., 1988; Preuss et al., 1996; Dancause et al., 2006a). In visually guided grasping movements, neuronal activity in PMv is initiated in the early, preparatory stages (Godschalk et al., 1985; Kurata and Wise, 1988) and neurons discharge selectively for specific types of hand configurations (Rizzolatti et al., 1988; Murata et al., 1997). Further supporting the key role of PMv in the visuomotor transformations for hand movements, transient inactivation of PMv in monkeys (Fogassi et al., 2001) and humans (Davare et al., 2006) generates deficits of hand pre-shaping for grasping.

One way PMv can participate in movement production is by modulating the activity of neurons in the primary motor cortex (M1) (Tokuno and Nambu, 2000; Kraskov et al., 2011) or the outputs of M1. Several studies using double or paired-pulse stimulation protocols have investigated the modulatory effects of the PMv ipsilateral to M1 on the outputs of M1 to intrinsic hand muscles of the contralateral arm. They showed that conditioning stimulations in the ipsilateral PMv (iPMv) can have both facilitatory and inhibitory effects, depending on the phase of movements or the configuration of the hand required for the task. For example, in humans at rest, PMv has inhibitory effects on M1 outputs to intrinsic hand muscles (Davare et al., 2008). During power grip, inhibitory effects are decreased and during precision grip, PMv becomes facilitatory. Furthermore, during the preparatory period prior to grasp, facilitatory effects of PMv are specific to the muscle that will be used (Davare et al., 2009).

However, hand configuration to grasp objects requires the coordinated activation of intrinsic hand as well as forearm muscles (Brochier et al., 2004) and the pattern of activity in these two muscles groups varies in function of the type of grasping movement being performed (Long et al., 1970). Accordingly, PMv may have different patterns of modulatory effects on intrinsic hand and forearm muscles in order to configure the hand into a desired shape. Supporting this hypothesis, recordings of cervical motoneurons from anaesthetized macaque monkeys have revealed that facilitatory effects from PMv conditioning are more frequent in intrinsic hand than forearm muscles (Shimazu et al., 2004). A more systematic comparison of the impact of PMv on outputs to intrinsic hand and forearm muscles would improve our understanding of its range of modulatory effects.

In addition to its involvement in the control of the contralateral hand, PMv is also active during ipsilateral movements. A large population of neurons in PMv discharge when monkeys perform tasks with either the ipsi or contralateral hand (Rizzolatti et al., 1988; Tanji et al., 1988) and in humans, sequential finger movements are associated with increased ipsilateral hemodynamic activity, most likely centered in PMv (Hanakawa et al., 2005). The extensive network of interhemispheric connections between PMv and M1 (Boussaoud et al., 2005; Dancause et al., 2007) could certainly allow the contralateral PMv (cPMv) to modulate M1 outputs. To date, transcranial magnetic stimulation (TMS) studies that have investigated interhemispheric interactions from contralateral premotor areas on M1 outputs have largely focused on the contralateral dorsal premotor cortex (PMd) (Mochizuki et al., 2004; Baumer et al., 2006; Koch et al., 2007; Liuzzi et al., 2010; Liuzzi et al., 2011). To our knowledge, no study has yet investigated the modulatory effects of cPMv on M1. Given the pattern of neural activity in cPMv during movements of the ipsilateral hand and the numerous interhemispheric connections of cPMv with M1, cPMv is likely to also have substantial modulatory effects on M1 outputs. A study of cPMv's modulatory effects would thus provide much needed insight into interhemispheric interactions from this premotor area on M1.

To address some of these issues, we conducted paired-pulse stimulation protocols using intracortical microstimulation techniques (ICMS) in sedated cebus monkeys. We compared the modulatory effects of a conditioning stimulus (C_{stim}) either applied to iPMv or cPMv at the same time as or prior to a test stimulus (T_{stim}) in M1. Modulatory effects of the C_{stim} were quantified in electromyographic (EMG) activity recorded in intrinsic hand and forearm muscles.

Methods

Subjects

Four adult female capuchin monkeys (*Cebus apella*) were used in this study (CB1: 1.9kg, CB2: 1.25kg, CB3: 1.4kg, CB4: 1.2kg). Monkeys were group housed and supplied with food and water ad libitum. The experimental protocol followed the guidelines of Canadian Council on Animal Care and was approved by the Comité de Déontologie de l'Expérimentation sur les Animaux (CDEA) of the Université de Montréal.

Surgical procedures

Data were collected in a terminal procedure. Anesthesia was induced with an intramuscular injection of 15 mg/kg of ketamine hydrochloride (Ketaset; Pfizer, Inc, New York, NY, USA). The animal was transitioned to ~2 % isoflurane (Furane; Baxter, Deerfield, IL, USA) in 100% oxygen and placed in ventral decubitus in a stereotaxic apparatus. To help prevent inflammation and swelling of the brain, the animal received an intramuscular injection of Dexamethasone 2 (Vetoquinol®; 0.5 mg/kg) and intravenous injection of Mannitol 20% (1500 mg/kg) at the beginning of the surgery. Proper hydration was maintained through a continuous intravenous infusion of lactated ringer's solution (10 ml/kg/h). The animal's body temperature was maintained near 36.5°C throughout the surgery using a homeothermic blanket (Harvard Apparatus, Holliston, MA). Blood oxygen saturation and heart rate were continuously monitored.

Figure 2.1A illustrates our experimental setup. Insulated, multistranded microwires (Cooner Wire, Chatsworth, CA, USA) were implanted intramuscularly for the recording of electromyographic (EMG) signals. For CB1, six muscles in each arm were implanted (*flexor pollicis brevis* (FPB)*, extensor carpi ulnaris* (ECU)*, extensor digitorium communis* (EDC)*, palmaris longus* (PL)*, biceps brachii* (BB) and *triceps brachii* (TB). For the other 3 monkeys, the same muscles were implanted as well as the *abductor pollicis brevis* (APB) and the *flexor digitorum superficialis* (FDS). Accurate placement of the EMG wires was confirmed by electrical stimulation of the muscle using the implanted wires and observation of the evoked movements.

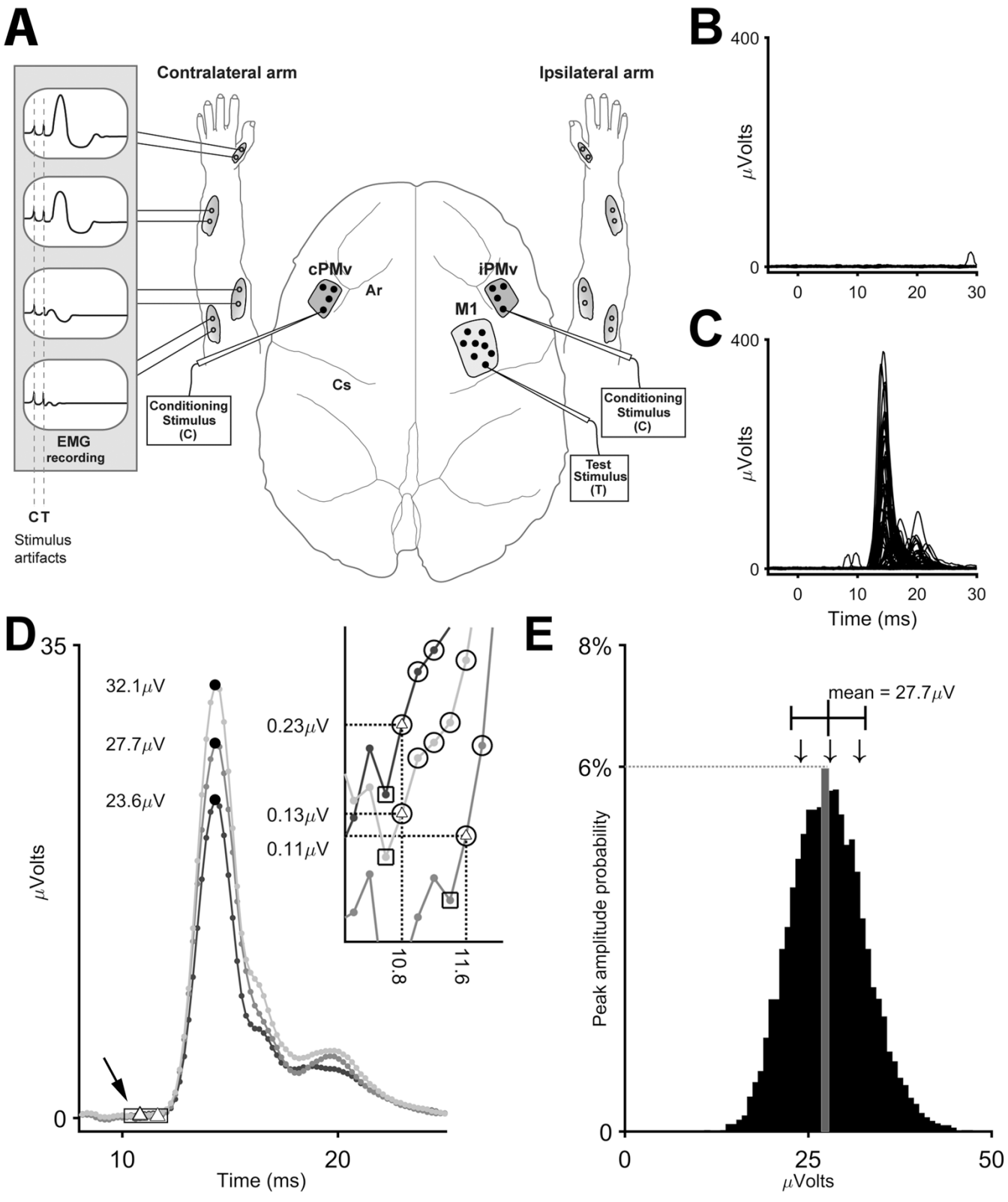
A
Contralateral arm
Ipsilateral arm
cPMv
Ar
iPMv
M1
Cs
Conditioning Stimulus (C)
Conditioning Stimulus (C)
Test Stimulus (T)
EMG recording
CT
Stimulus artifacts
B
400
μVolts
0
0
10
20
30
C
400
μVolts
0
0
10
20
30
Time (ms)
D
35
32.1μV
27.7μV
23.6μV
0.23μV
0.13μV
0.11μV
10.8
11.6
μVolts
0
10
20
Time (ms)
E
8%
6%
mean = 27.7μV
Peak amplitude probability
0
0
25
50
μVolts

Figure 2.1 *Experimental methods.*

A) Schematic representation of the experimental setup. Six (n=1) or 8 muscles (n=3) in each arm were implanted in each monkey to record EMG signals. We located M1 (light gray area) and iPMv in one hemisphere and cPMv in the opposite hemisphere (dark gray areas). Dots within the shaded areas show hypothetical stimulation sites in these cortical areas. The two electrodes used for the paired-pulse protocol were then positioned in hand representations of M1 of iPMv or cPMv. Ar: arcuate sulcus; Cs: central sulcus. **B)** Example of single trial responses in the flexor pollicis brevis (FPB) with the C-only condition (n=150) applied in iPMv in a representative protocol. The current intensity of conditioning stimulus (C_{stim}) was adjusted to be subthreshold. Accordingly, no obvious MEP is observed. **C)** Single trial responses in the FPB with the T-only stimulation in M1 (n=150) in the same protocol. The current intensity for the delivery of the single pulses was set at 125% of the threshold, yielding a clear MEP. **D)** From the same protocol, 3 average predicted MEPs calculated with C-only and T-only trials are shown. Each average predicted MEP was generated by averaging 150 randomly drawn predicted traces from the pool of all C-only and T-only combinations (see methods). For each average predicted motor evoked potential (MEP), the peak maximum (black dots) and minimum (white triangles) are identified. The inset (top right) is a magnified view of the peak minima obtained using a backward march from the peak (small black arrow). Circles indicate points with a voltage value at 5% higher than the following points on the backward march (see Methods). Squares indicate the first point with a voltage value less than 5% higher than the following point on the backward march. The peak minimum was the previous point (triangle within a black circle). The peak amplitude was defined as the change in potential between the peak minimum and the peak maximum. This process was repeated 10,000 times to produce the probability distribution of predicted peak amplitudes shown in E. **E)** Histogram of the probability distribution of predicted peak amplitudes in the FPB for the same protocol. The histogram presents the probability of occurrence (y axis) of peaks with different magnitudes (x axis). For example, the highlighted bin in gray shows that the probability that the average predicted MEPs would have amplitudes between 27.3 and 28 μV is approximately 6%. The black line and whiskers above the histogram indicate the mean and standard deviation of the distribution. The arrows indicate the location of the peak amplitudes from the 3 example traces in panel D. To quantify the interaction effects in paired-pulse trials (C+T), the conditioned MEP peak amplitude Z-score was compared to this probability distribution.

Once the EMG electrodes were implanted, craniotomies and durectomies were performed to expose the primary motor cortex (M1) in one hemisphere as well as both the ipsilateral and contralateral ventral premotor areas (iPMv, cPMv respectively).

Paired-pulse stimulation and EMG recording

At the end of the surgical procedures, gas anesthesia was turned off and the animal was kept deeply sedated with intravenous injections of ketamine (~10 mg/kg/10 minutes) and Diazepam (Valium; 0.01mg/kg/hr) for electrophysiological data collection. In order to facilitate the search for suitable stimulation sites to use in the paired-pulse protocols, we first located the hand representation in M1, iPMv and cPMv using standard ICMS trains (Mansoori et al., 2014; Deffeyes et al., 2015; Dea et al., 2016; Touvykine et al., 2016). All cortical sites retained for the paired-pulse protocols evoked clear digit or wrist movements in the contralateral arm with ICMS trains.

Two glass-coated tungsten microelectrodes (~1 MΩ impedance; FHC Bowdoin, ME USA) were used for the paired-pulse stimulations. They were lowered perpendicular to the cortex with a micromanipulator to depths of ~1800μm (layer V) below the surface. The electrode for T_{stim} was positioned in M1 of the right hemisphere with a micromanipulator. The electrode for the C_{stim} was placed in either the iPMv (right hemisphere) or cPMv (left hemisphere) with a second manipulator (see Figure 2.1A). Both the C_{stim} and T_{stim} were cathodal single square pulses of 0.2ms duration. The stimulation intensities for the C_{stim} and T_{stim} were determined independently online, based on evoked EMG activity in muscles of the arm contralateral to the stimulation. If EMG activity was present in more than one muscle, the muscle with the lowest threshold was used to determine the desired current intensity. The intensity for the C_{stim} was set at 75% of the EMG threshold (range = 95 - 225 μA, mean = 197 μA). If no EMG response could be observed with up to 300μA, the intensity of the C_{stim} was arbitrarily set to 225 μA. The current intensity used for the T_{stim} in M1 was typically set to 125% of threshold (range = 40 - 300 μA, mean = 170 μA). In some cases, if the evoked activity was too small or too big with this value, the intensity was adjusted to a level producing clear but submaximal response.

Once the locations of the 2 electrodes and the proper stimulation intensities were selected, a paired-pulse stimulation protocol was initiated. In a protocol, stimulations could be delivered through the conditioning electrode only (C-only), the test electrode only (T-only), or through both with 6 different inter-stimulus intervals (ISIs). When the C_{stim} was in iPMv, the paired stimulations (C+T) could be delivered simultaneously (ISI0) or with C_{stim} preceding the T_{stim} by 1ms (ISI1), 2ms (ISI2), 4ms (ISI4), 6ms (ISI6) or 10ms (ISI10). When the C_{stim} was in cPMv, we presented both stimulations simultaneously (ISI0) or with ISIs of 2.5ms (ISI2.5), 5ms (ISI5), 10ms (ISI10), 15ms (ISI15) or 20ms (ISI20). A total of 150 trials per condition were collected (8 conditions per protocol; total stimulations = 900). For monkeys CB1 and CB2, data for each condition were recorded in three blocks of 50 trials delivered at 3Hz and the stimulation condition of subsequent blocks was randomized (Deffeyes et al., 2015). For monkeys CB3 and CB4, the condition of each subsequent trial was randomly selected until a total of 150 trials delivered at 3Hz for each condition was collected. We confirmed that the responses were stable across the recording. For all recorded protocols, we performed a two-sample t-test and verified that the response evoked with the T-only from the first 75 trials was not different to the response from the last 75 trials ($t = -0.68$; $p=0.50$).

After completion of data collection for a protocol, the two electrodes were moved to different cortical locations and another protocol was initiated. In the 4 monkeys, we collected a total of 22 protocols, 11 with the C_{stim} electrode in iPMv and 11 with the C_{stim} electrode in cPMv. As EMG signals were simultaneously recorded from 6 (n=1) or 8 muscles (n=3), we thus collected 164 EMG signals under 8 conditions yielding 656 recordings for iPMv conditioning and 656 for cPMv conditioning.

Both the paired-pulse stimulations and EMG data recording were controlled with an RZ5 real-time processor (Tucker Davis Technologies (TDT), Alachua, FL, USA) running custom software designed for this procedure. Part of the software controlled the stimulations that were produced by an IZ2 stimulator (Tucker Davis Technologies (TDT), Alachua, FL, USA). Another part controlled the data acquisition. Each EMG channel was recorded at 4.9 kHz. Raw EMG data were stored for offline processing.

Electromyographic (EMG) data analysis

Offline data analyses were done using custom written MatLab (Version R2014a; Nantick, MA, USA) code. The continuously recorded raw EMG data were separated into individual trials and aligned to the end of the C_{stim} for the C-only condition, and to the end of the T_{stim} for the T-only and for the 6 paired-pulse conditions. The EMG signal in a window of 30ms after the end of the stimulation was analyzed. The raw EMG was full-wave rectified, and smoothed using a 5-point moving average (window = 1.02ms). Note that no additional filters were used to remove the stimulus artifacts. Traces presented show the extent of the artifact, when present, along with the EMG responses.

For each of the 164 EMG signals, we first established if the T_{stim} evoked a detectable motor evoked potential (MEP) (T-only condition) and that this response was large enough for us to detect either increases or decreases of activity by the C_{stim}. To do this, the T-only trials were averaged and the MEP response was compared to the baseline activity in the 30ms prior to the first stimulus. If the average MEP peak amplitude was greater than 3 standard deviations (SD) above the average baseline, it was considered significant and kept for subsequent analyses.

In the present study, we focused our analyses on the modulation of peak amplitude by the C_{stim}. For each significant average MEP evoked with the T_{stim} only, the first step was to generate a population of predicted responses based on the summation of responses in C-only and T-only trials (Figure 2.1B-E). We performed all possible combinations of single C-only traces (n=150) with single T-only traces (n=150) and linearly summed them to produce predicted traces (n=22,500). Because the target current intensity for the C_{stim} was sub-threshold, the major contribution of these combined responses are from the T_{stim}. However, we preferred the predicted MEPs to account for any potential small EMG response from the C_{stim} that may have occurred over many trials (Deffeyes et al., 2015). Out of the population of predicted traces, we randomly drew samples of 150 trials and averaged them to produce average predicted MEPs (Figure 2.1D). For each average predicted MEP, we calculated the peak amplitude according to the following formula:

$$Peak\ amplitude = peak\ maximum - peak\ minimum$$

where the MEP *peak maximum* is defined as the maximum voltage value within a 30ms window after the end of the stimuli and the *peak minimum* is the voltage value at the peak onset time. Our algorithm searched for the peak onset from a point clearly within the peak (10% of the peak

maximum voltage) and marching back toward the beginning of the trial (time 0). The voltage value of each data point was compared to the one of the next point on that backward march. The first point with a voltage value of less than 5% higher than the following point was considered as not being part of the response and thus the previous point in the backward march was defined as the peak onset time (Figure 2.1D). We chose this approach, instead of simply using pre-stimuli baseline for example, because we found it yielded more accurate results. This was especially obvious when the signal was small in comparison to baseline, something that often occurred when the conditioning stimulus had inhibitory effects (see results).

This process was repeated 10,000 times to generate a probability distribution of predicted peak amplitudes (Figure 2.1E)(Stanford et al., 2005). This probability distribution describes the range of responses that could be obtained if there were no interactions between neurons stimulated by the C_{stim} and T_{stim} electrodes. Then, responses of all trials with the paired-pulse (C+T) with each ISI were averaged (n=150) and the MEP peak amplitude was obtained similarly as described above. The responses obtained when conditioning iPMv or cPMv with the different ISIs were compared to the probability distribution to evaluate the direction (facilitation, inhibition or no modulation) and the normalized strength of modulatory effects from PMv on M1 output by calculating the Z-score of the MEP peak amplitude (Figure 2.2). The modulation of M1 output by PMv conditioning was deemed significant when the Z-score of a C+T MEP peak amplitude differed by more than 1.96 SD from the mean of the distribution of predicted peak amplitudes ($p \leq 0.05$). Consequently, an MEP peak amplitude Z-score value ≤ -1.96 was considered a significant inhibition while a Z-score value ≥ 1.96 was considered a significant facilitation.

Although we collected a limited number of cortical sites per area in each animal (n=2-4), we verified that the general modulatory effects of iPMv and cPMv conditioning were comparable across monkeys. An ANOVA comparing the peak amplitude of the MEPs across monkeys showed no significant difference for either iPMv ($F=2.4$; $p=0.83$) or cPMv ($F=1.1$; $p=0.36$) conditioning. It is also worth noting that because stimulations are applied at the cortical level and effects are recorded in the EMG signals, these techniques do not provide clear information about the locus of interactions, which may occur at the cortical level but also at other places along the neural axis.

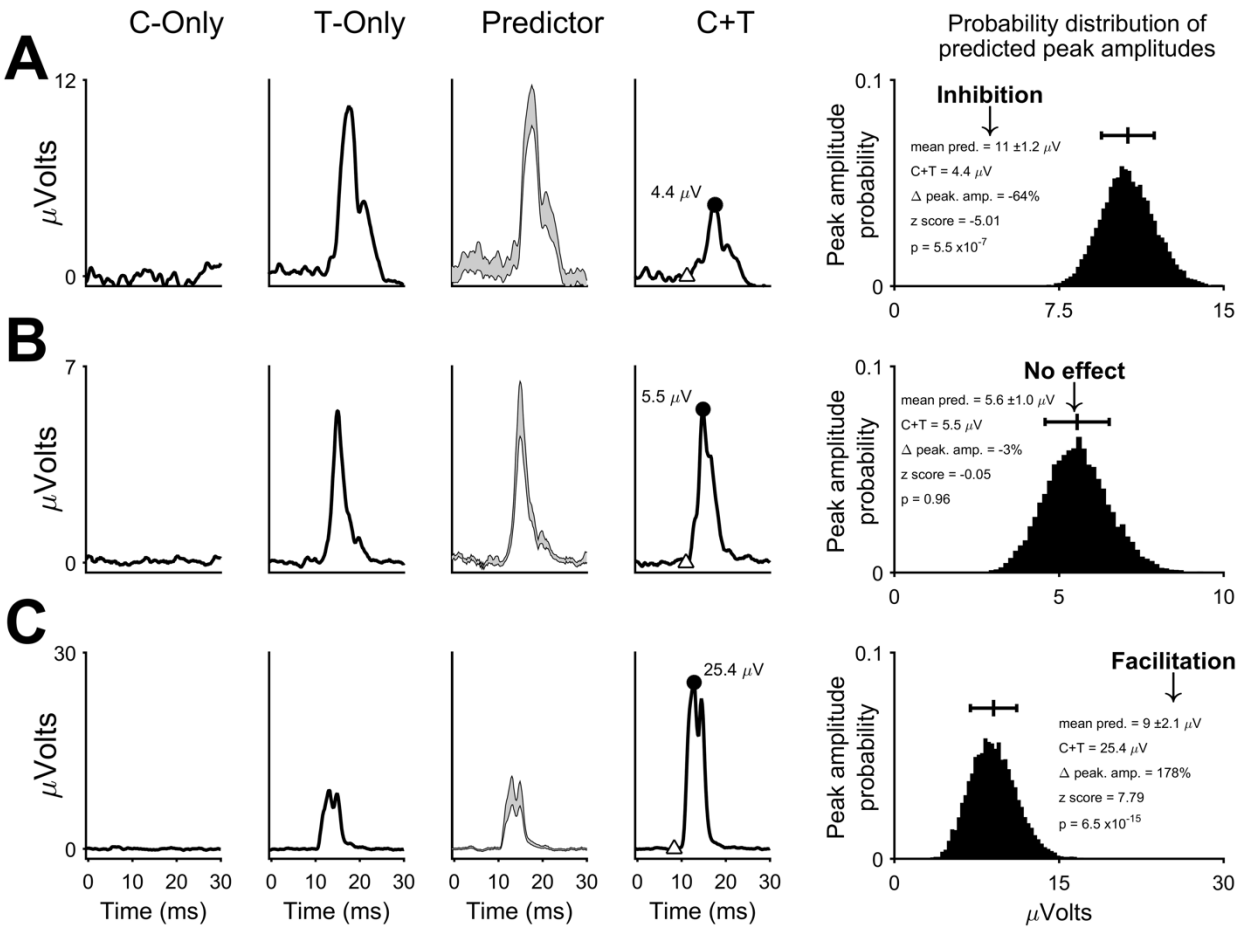

Figure 2.2 *Comparison of the conditioned response with the probability distribution*

Each row shows an example of comparison between the probability distribution of predicted peak amplitudes and a conditioned response (C+T). EMG traces are aligned (Time = 0ms) to the end of the C_{stim} for the C-only trials and to the end of the T_{stim} for the T-only and the C+T trials. **A)** The top row shows an example in which the conditioning of the contralateral PMv (cPMv) decreased the MEP of the FPB (inhibitory effect). The first and second columns show mean traces of 150 trials with C-only and T-only stimulations, respectively. To provide an appreciation of the variability of the predicted responses, the third column shows +/- one standard deviation of the mean of all 10,000 average predicted MEPs. The fourth column shows the mean MEP when the C_{stim} preceded the T_{stim} by 15ms (ISI15). The conditioned MEP peak maximum (black dot) and minimum (white triangle) values were identified to calculate the peak amplitude. In the fifth column, the relative value (Z-score) of the conditioned MEP peak amplitude (arrow) is compared to the probability distribution of predicted peak amplitudes. MEP peak amplitude Z-scores ≤ -

1.96 or ≥ 1.96 were considered significantly different from the prediction ($p \leq 0.05$). The black line and the whiskers above the histogram of the probability distribution show its mean and standard deviation, respectively. In this example, the conditioned MEP was smaller and its peak amplitude of 4.4 μV is clearly outside the range of the predicted peak amplitudes. This translates into a strongly significant negative Z-score ($Z=-5.1$; $p=5.5 \times 10^{-7}$). Thus, with ISI15 the conditioning of cPMv resulted in a significant inhibition of M1 outputs to this muscle. **B)** The middle row shows an example in which the conditioning of iPMv 1ms before the T stimulation in M1 (ISI1) had no effect on the MEP recorded in APB. The conditioned MEP (4th column) is not markedly different from the mean predicted MEPs (3rd column). Accordingly, the 5.5 μV value of the peak amplitude of the conditioned MEP (5th column, arrow) fell within the range of the predicted peak amplitudes and the Z-score was not significant ($Z=-0.05$; $p=0.96$). This result supports that with ISI1, the C stimulation in iPMv did not modulate M1 output to this muscle. **C)** The bottom row shows an example in which the conditioning of iPMv 10ms before the T stimulation (ISI10) increased the MEP recorded in FDS. The conditioned MEP (4th column) is much greater than the mean predicted MEPs (3rd column). Its peak amplitude of 25.4 μV is clearly outside the range of the predicted peak amplitudes (5th column, arrow) with a strongly significant positive Z-score ($Z=7.79$; $p=6.5 \times 10^{-15}$). Thus, with ISI10 the C stimulus in iPMv significantly facilitated M1 output to this muscle.

Results

We conducted a total of 22 paired-pulse protocols in 4 cebus monkeys. Figure 2.3 shows the cortical location of the C_{stim} and T_{stim} electrodes for these protocols in relation to cortical vasculature and sulci as well as movements evoked with ICMS trains. Mapping was more extensive in M1 to provide some information about the extent of the hand representation. Additional mapping was done in the opposite hemisphere to locate the cPMv hand representation. The iPMv was then easily located by stimulating cortical sites in the homotopic area in the ipsilateral hemisphere. For both the C_{stim} and T_{stim} electrodes, all cortical sites retained for the paired-pulse protocols evoked clear digit or wrist movements in the contralateral arm with ICMS trains. As such, our study focuses on interactions of outputs from cortical areas involved in the generation of distal forelimb movements.

For each of the 22 protocols, the T-only condition evoked a significant MEP (>3 SD above baseline; see Methods) in at least one and up to 7 muscles of the contralateral arm (total = 87 MEPs). These MEPs were more common in the FPB (n=22), ECU (n=17), APB (n=16) and EDC (n=14). They were less common in FDS (n=9) and PL (n=8). Only 1 T_{stim} site in M1 induced a clear MEP in BB and none produced MEPs in TB. This is not surprising as we specifically placed our C_{stim} and T_{stim} electrodes at cortical sites that evoked digit or wrist movements with ICMS trains. The overall mean onset latency for all muscles was 14.87±2.5ms (mean ± SD). Because only one MEP was found in the BB we excluded it from further analyses. Comparing latencies of the MEPs evoked with T-only trials, a one-way ANOVA confirmed that there was a main effect of muscle ($F=8.93$, $p<0.01$). Post hoc pairwise comparisons using Bonferroni method to correct for multiple comparisons confirmed that the MEPs in the two intrinsic hand muscles (APB and FPB) had similar latencies ($p>0.05$; combined mean = 16.6±2.2ms), which were significantly longer ($p<0.001$) than those of MEPs in forearm muscles (combined mean = 13.5±1.7ms). There was no difference in MEP latencies between forearm muscles ($p>0.05$).

We analyzed the effects of iPMv and cPMv conditioning on the MEPs evoked in intrinsic hand and forearm muscles. Figure 2.4 shows different examples of modulations of the MEP with the various ISIs used in our protocols for both iPMv (Figure 2.4A-C) and cPMv (Figure 2.4D-F) conditioning. For some cortical sites, when the conditioning stimulation had an effect, the peak

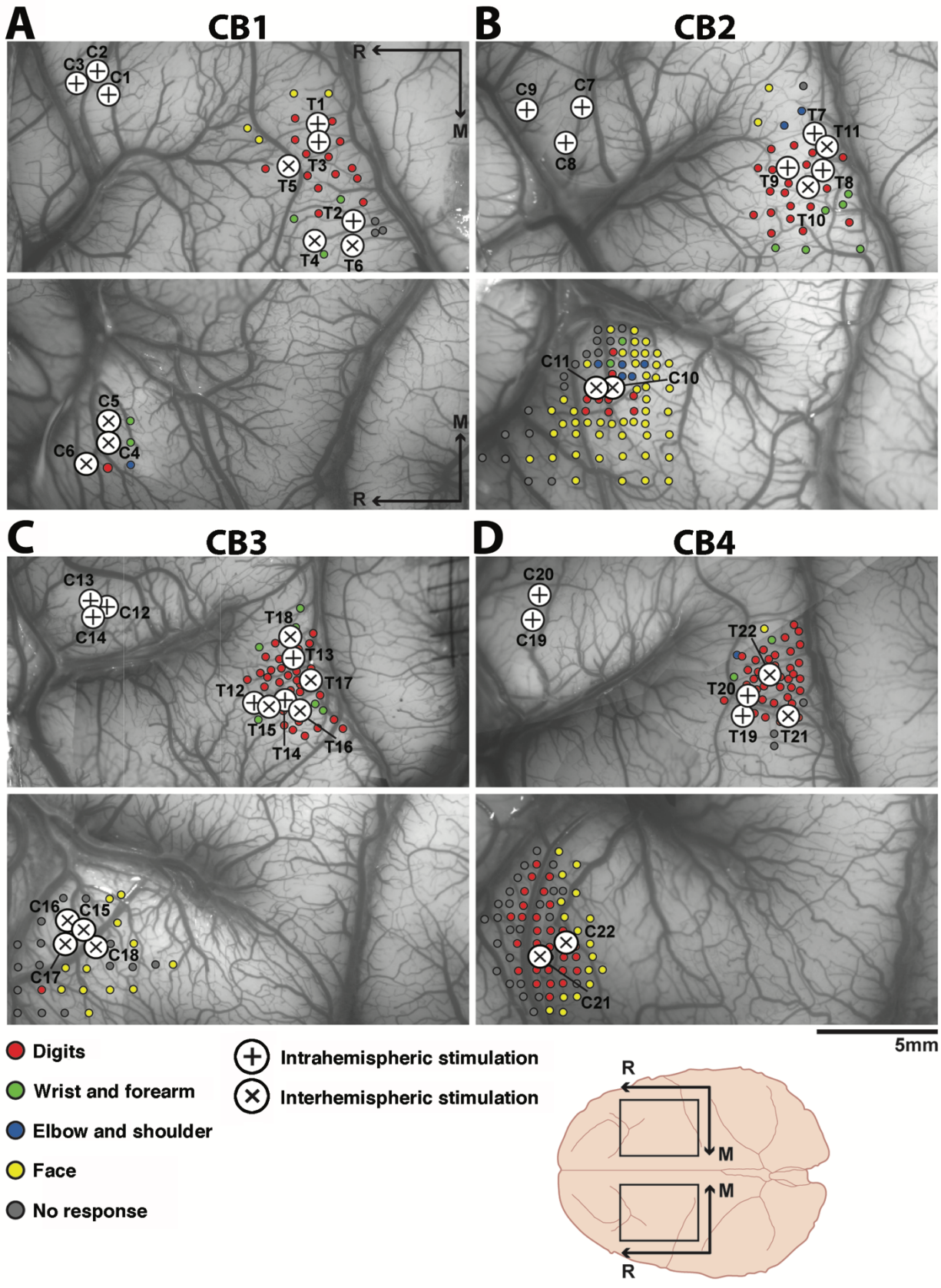
A
CB1
C2
C3
C1
R
M
T1
T3
T5
T2
T4
T6
C5
C6
C4
M
R
B
CB2
C9
C7
C8
T7
T11
T9
T8
T10
C11
C10
C
CB3
C13
C12
C14
T18
T13
T12
T17
T15
T14
T16
C16
C15
C18
C17
D
CB4
C20
C19
T22
T20
T19
T21
C22
C21
5mm
Digits
Wrist and forearm
Elbow and shoulder
Face
No response
Intrahemispheric stimulation
Interhemispheric stimulation
R
M
M
R

Figure 2.3 *Cortical location of the Cstim and Tstim electrodes selected for paired-pulse protocols*

A) Motor mapping data and cortical sites selected for the paired-pulse protocols conducted in CB1. ICMS trains were used to locate the hand representation in M1 and in cPMv (colored dots). The evoked movements in the forearm and hand muscles contralateral to the stimulated hemisphere at threshold current intensity are color-coded according to the legend at the bottom of the figure. Once the hand representations were located, cortical sites evoking EMG responses in at least one of the implanted forearm or intrinsic hand muscles at relatively low current intensity were selected for the paired-pulse protocols. In CB1, 3 protocols were conducted with the C electrode in iPMv (large circles with +) and 3 protocols with the C_{stim} electrode in cPMv (large circles with ×). The location of the T electrodes in M1 for each protocol is shown with the same symbols. **B)** Motor mapping data and cortical sites selected for the paired-pulse protocols conducted in CB2. In this animal, 3 protocols were conducted with the C_{stim} electrode in iPMv and 2 protocols with the C_{stim} electrode in cPMv. **C)** In CB3, 3 protocols were conducted with the C_{stim} electrode in iPMv and 4 protocols with the C_{stim} electrode in cPMv. **D)** Finally in CB4, 2 protocols were conducted with the C_{stim} electrode in iPMv and 2 protocols with the C_{stim} electrode in cPMv.

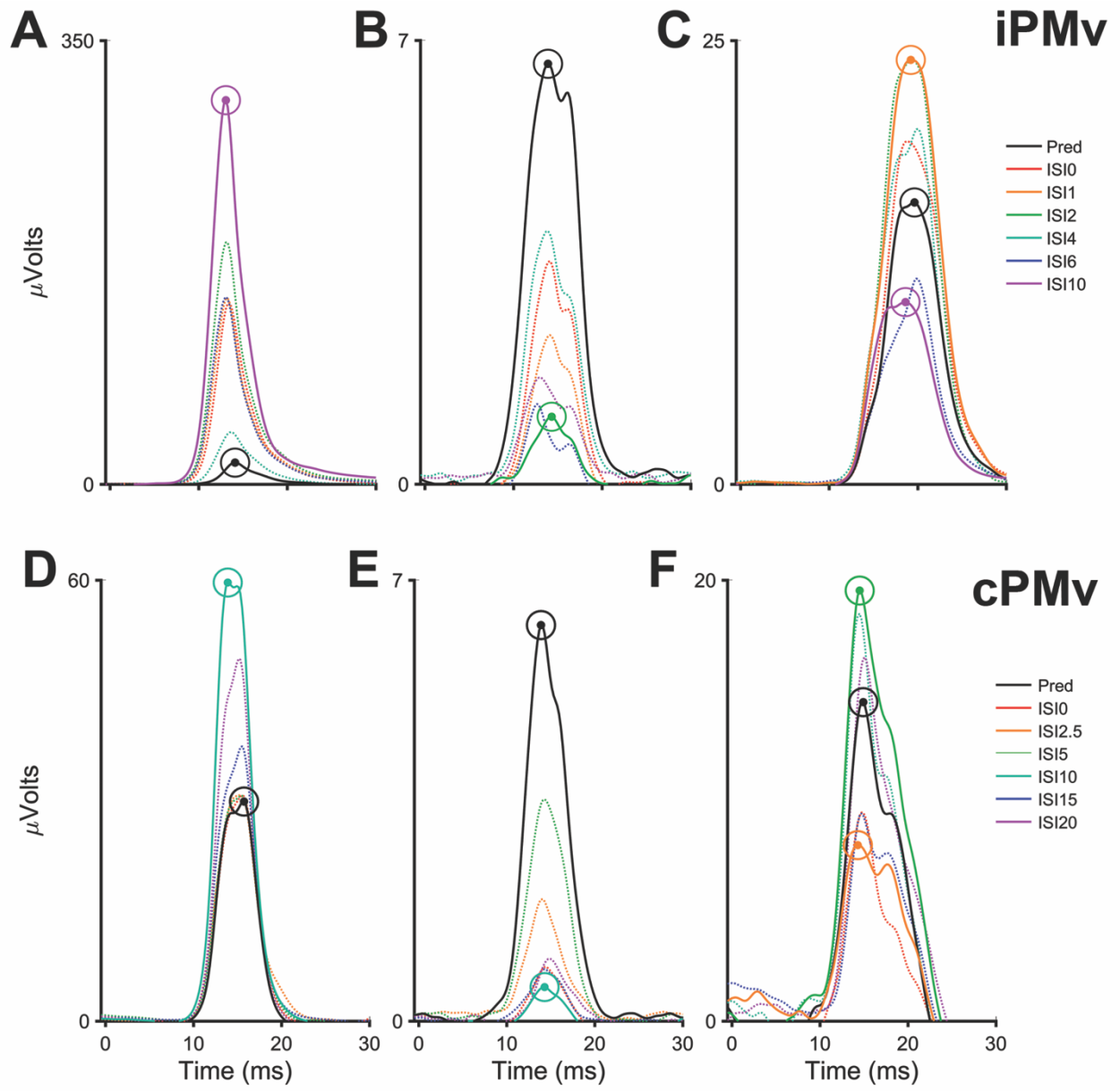

Figure 2.4 *Examples of modulatory effects caused by iPMv and cPMv conditioning*

The top row **(A-C)** shows examples in which the conditioning electrode was in iPMv and the bottom row **(D-F)** shows examples in which the conditioning electrode was in cPMv**.** Each panel presents MEPs in one muscle resulting from the different stimulation conditions in a protocol. The black line shows the mean of the 10,000 average predicted MEPs (see Figure 2.1D) calculated from the T-only and C-only trial. The colored lines show the average conditioned MEPs (C+T) obtained with the different ISIs, according to the legend on the right. **A)** We found cases in which the conditioning of iPMv produced a facilitation of the MEP. This example shows MEPs from the

FPB. The traces of conditioned MEPs all have greater peak intensities than the predictor, with ISI10 producing the most powerful facilitation (magenta curve). **B)** There were also cases in which the conditioning of iPMv inhibited the MEP. In this example, EMG was recorded from the FDS. The peak amplitude of the MEP after iPMv conditioning is smaller than the predictor with all ISIs. A delay of 2ms between the C_{stim} and T_{stim} (ISI2; green curve) produced the strongest inhibition. **C)** Finally, we found cases in which the conditioning of iPMv produced an inhibition of the MEP with some ISIs and a facilitation with others. The figure shows MEPs recorded from the APB. In this case, the MEP was larger than the predictor when iPMv was conditioned with short ISIs (e.g. orange curve: ISI1) and smaller when iPMv was conditioned with long ISIs (e.g. magenta curve: ISI10). **D)** Example of an MEP recorded in FPB that was facilitated by the conditioning of cPMv. **E)** Example of an MEP from the FDS that was inhibited by the conditioning of cPMv. **F)** Example of an MEP in FPB that was facilitated by the conditioning of cPMv with some ISIs and inhibited with others.

amplitude of the MEPs recorded with the C+T trials was greater than the mean predicted response regardless of the ISI (Figure 2.4A, D). For other cortical sites, the peak amplitude of the MEPs recorded with the C+T trials was smaller than the predicted response (Figure 2.4B, E). Finally, there were also cases in which the conditioning of iPMv or cPMv could have an inhibitory effect with some ISIs and a facilitatory effect with other ISIs (Figure 2.4C, F).

All individual MEPs collected in our experiments are presented as an intensity plot in Figure 2.5. In general, the T-only trials produced a clear response while the C-only did not evoke any MEP. The plot shows the MEPs with the different ISIs normalized to the peak value of the MEPs obtained with the T-only condition. As indicated by the frequent dark red areas, the conditioning in iPMv (Figure 2.5A) led to strong facilitation of the MEPs and these occurred much more often in intrinsic hand (top row) than in forearm muscles (bottom row). Conditioning of cPMv (Figure 2.5B), as indicated by the common blue areas, led more often to inhibition of MEPs in both intrinsic hand and forearm muscles.

Effects of iPMv conditioning on MEPs in intrinsic hand and forearm muscles

For protocols in which we applied the C_{stim} in iPMv, the T-only condition induced a total of 19 MEPs in intrinsic hand muscles (APB=8; FPB=11) and 23 MEPs in forearm muscles (ECU=9; EDC=8; FDS=3; PL=3). For intrinsic hand muscles, when conditioning of iPMv modulated the outputs of M1, most often it was facilitatory (Figure 2.6A; white bars). Across studied ISIs, of the 74 significant modulation of MEPs we found, 62 were facilitatory (83.8%) and they were most common when the C_{stim} preceded the T_{stim} by 1ms (ISI1; n=13), 2ms or 4ms (ISI2 and ISI4; n=12). Facilitatory effects were least common when the C_{stim} and T_{stim} were delivered simultaneously (n=7). We also studied the magnitude of the modulation of M1 outputs produced by iPMv conditioning using the relative measure of the intensity of modulatory effect (Z-score) (Figure 2.6B). Note that in order to give a more faithful representation of the intensity of the modulatory effect of the conditioning pulse, we used all intrinsic hand muscle MEPs for this analysis and not only the MEPs significantly modulated by the conditioning stimulus with the different ISIs (Figure 2.6A). This analysis shows that facilitation tended to be most powerful with ISI10. Strong

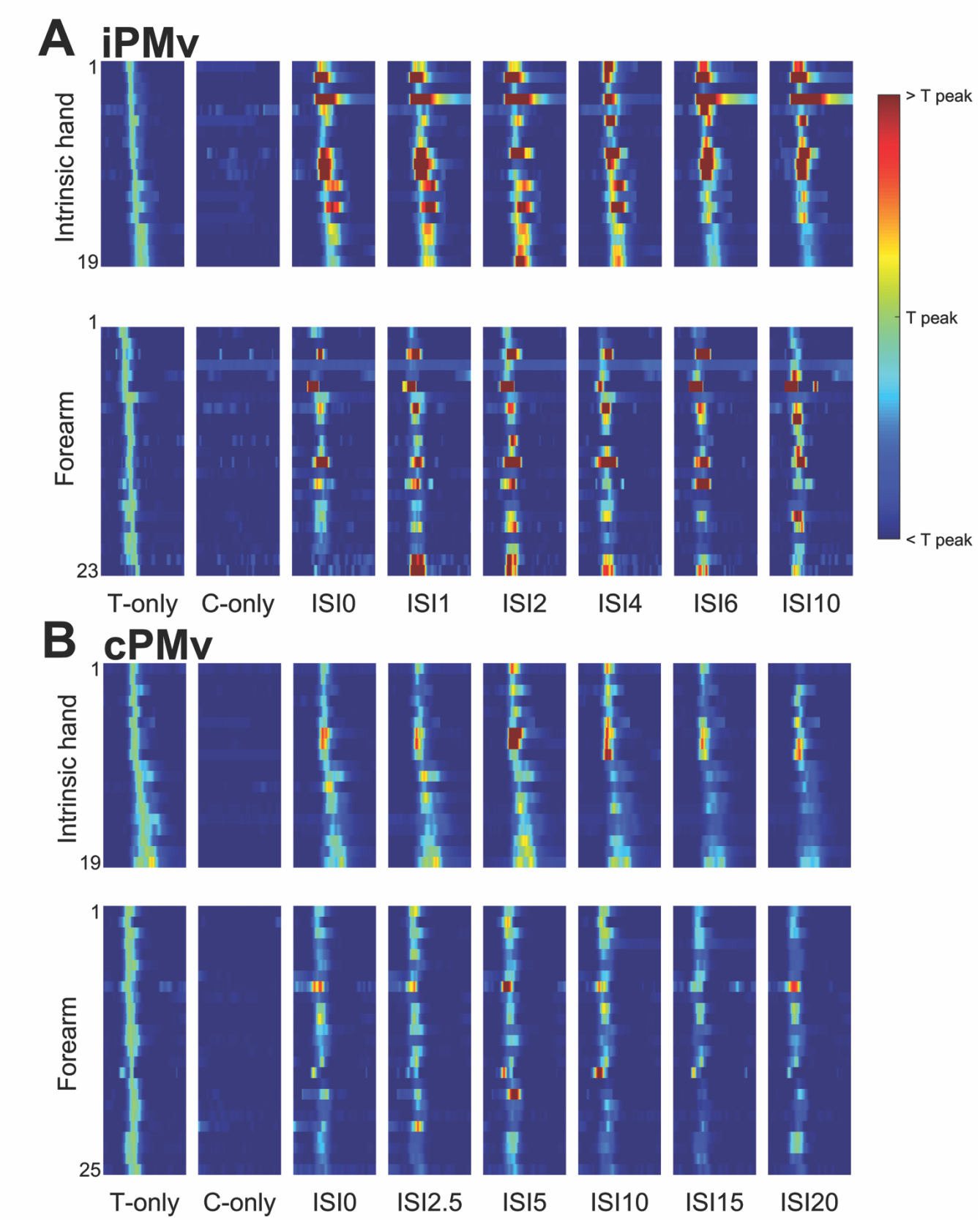
A
iPMv
Intrinsic hand
1
19
Forearm
1
23
T-only
C-only
ISI0
ISI1
ISI2
ISI4
ISI6
ISI10
> T peak
T peak
< T peak
B
cPMv
Intrinsic hand
1
19
Forearm
1
25
T-only
C-only
ISI0
ISI2.5
ISI5
ISI10
ISI15
ISI20

Figure 2.5 *Complete data set of modulatory effects of PMv conditioning on M1 outputs*

A) MEPs conditioned with iPMv stimulation. The top row shows the 19 MEPs recorded from the intrinsic hand muscles (FPB and APB) and the bottom row the 23 MEPs recorded from the forearm muscles (ECU, EDC, PL, FDS) in the 4 monkeys. The different columns, from left to right, show the responses evoked with the T-only, C-only and the 6 different ISIs. Individual rows within the intensity plot show individual MEPs recorded over a period of 40ms starting at the end of the stimulus (time = 0). The recordings are ordered based on peak amplitude of the MEP with the T-only trials, and kept for all conditions. The color scale on the right indicates the range of responses normalized to the MEP peak amplitude with the T-only stimulation. Accordingly, in the $C+T_{stim}$ trials with the different ISIs, traces in the yellow-red range indicate facilitation of the MEP with the conditioning stimulus and traces in the light to dark blue range indicate inhibition. The common dark red colors support that iPMv conditioning induced strong facilitation of the MEPs, more often in intrinsic hand than in forearm muscles. **B)** MEPs conditioned with cPMv stimulation. The top row shows the 19 MEPs recorded from the intrinsic hand muscles and the bottom row the 25 MEPs recorded from the forearm muscles. In comparison to conditioning in iPMv, light to dark blue colors are more frequent. This supports that the conditioning of cPMv induced more inhibitory effects in both intrinsic hand and forearm muscles than iPMv.

facilitations were also evoked with ISI2 and ISI1. Overall, this pattern of facilitation across ISIs is quite similar to what has previously been described in sedated macaque monkeys (Cerri et al., 2003).

We also found incidences of significant inhibitory effects with some tested ISIs (Figure 2.6A; black bars). These inhibitory effects were much less common than facilitatory effects and represented only 16.2% of the significant modulations (n=12). They were more likely to occur when the C_{stim} preceded the T_{stim} by longer delays (ISI6 n=5 and ISI10 n=4). When the C_{stim} in iPMv preceded the T_{stim} in M1 by 2 or 4ms, we found no significant inhibitory effects. The magnitude of inhibitory effects from iPMv conditioning on intrinsic hand muscles (Figure 2.6B; black bars) was also much weaker in comparison to facilitatory effects. Inhibitory effects tended to be slightly more powerful with longer ISIs. Together, these data support that for intrinsic hand muscles involved in thumb movements, iPMv is much more likely to have facilitatory than inhibitory effects on M1 outputs and the facilitatory effects are much more powerful.

Modulatory effects of iPMv conditioning on forearm muscles were quite different. Across all ISIs, only 23 cases of significant modulations were facilitatory (33.8%) and their incidence increased with longer ISIs (Figure 2.6C). The magnitude of the facilitation induced by iPMv conditioning was also much smaller for forearm muscles (Figure 2.6D) and tended to increase with longer ISIs. Significant inhibitory effects were twice as common as facilitatory effects (n=45; 66.2%). Most cases of inhibitory effects were found with ISI6 (n=10) but many were found with all tested ISIs. The magnitude of the inhibitory effects on forearm muscles induced by iPMv conditioning did not vary much across ISIs but it was slightly more powerful when the C_{stim} preceded the T_{stim} by 6ms (ISI6). In contrast to intrinsic hand muscles, inhibitory and facilitatory effects in forearm muscles were of comparable magnitude. Overall these results support that iPMv is more likely to have inhibitory than facilitatory effects on forearm muscles and that the magnitude of the facilitatory effect on forearm muscles is weaker than on intrinsic hand muscles.

Effects of cPMv conditioning on MEPs of intrinsic hand and forearm muscles

For protocols where cPMv was the source of conditioning, we found a total of 19 significant MEPs

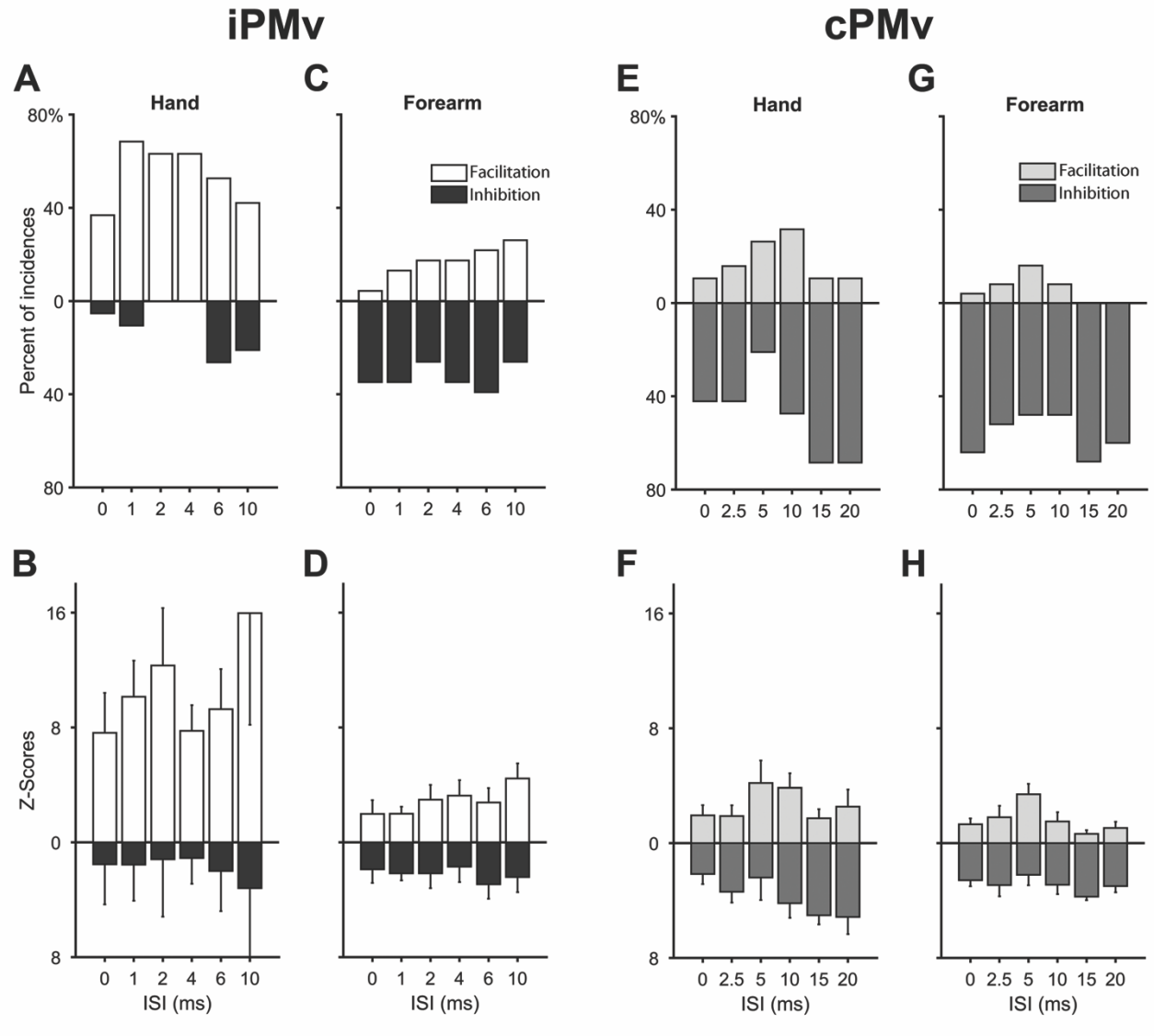

Figure 2.6 *Effects of iPMv and cPMv conditioning on MEPs in intrinsic hand and forearm muscles*

A) Incidence of significant modulation of MEPs in the intrinsic hand muscles produced by iPMv. The histogram shows the proportion of the 19 MEPs that were significantly facilitated (white) or inhibited (black) with each ISI. For example, when both the C_{stim} and T_{stim} were applied simultaneously (ISI0), 7 of the 19 MEPs (36.8%) had a significant increase of peak amplitude in comparison to the predicted peaks (conditioning considered facilitatory) and only 1 (5.2%) had a significant decrease of peak amplitude (conditioning considered inhibitory). **B)** Magnitude of the modulation of MEPs in intrinsic hand muscles produced by iPMv conditioning. The histogram presents the mean (±SD) of the positive and negative Z-scores with each ISI. Facilitatory effects resulting from iPMv conditioning were also much more powerful than inhibitory effects. Note that

although there were no cases of significant inhibition with ISI2 and ISI4, because all 19 MEPs are used for this analysis, there is still a small Z-score value for inhibitory effects with these ISIs. **C)** Incidence of significant modulation of MEPs in the forearm muscles produced by iPMv. In contrast to intrinsic hand muscles, iPMv conditioning most often had inhibitory effects on MEPs. **D)** Magnitude of the modulation of MEPs in forearm muscles produced by iPMv conditioning. The magnitude of facilitatory effects was much smaller in forearm muscles than in intrinsic hand muscles. **E)** Incidence of significant modulation of MEPs in the intrinsic hand muscles produced by cPMv. The conditioning of cPMv is much more likely to have inhibitory effects on M1 outputs to intrinsic hand muscles. **F)** Magnitude of the modulation of MEPs in intrinsic hand muscles produced by cPMv conditioning. Apart from ISI5, inhibitory effects were more powerful than facilitatory effects for all other tested ISIs. **G)** Incidence of significant modulation of MEPs in the forearm muscles produced by cPMv. The predominance of inhibitory effects of cPMv conditioning was even greater for forearm than intrinsic hand muscles. **H)** Magnitude of the modulation of MEPs in forearm muscles produced by cPMv conditioning. Apart from ISI5, the magnitude of facilitatory effects was also smaller than inhibitory effects in forearm muscles.

in intrinsic hand muscles (APB=8; FPB=11) and 25 MEPs in forearm muscles (ECU=8; EDC=6; FDS=6; PL=5) with the T-only trials. For MEPs in intrinsic hand muscles, out of the significant modulations, cPMv conditioning facilitated M1 outputs in only 20 cases (26.7%), more of them occurring when the C_{stim} preceded the T_{stim} by 10ms (ISI10; n=6) or 5ms (ISI5; n=5) (Figure 2.6E; light gray bars). The magnitude of the facilitatory effect was also greater with these two ISIs (Figure 6F). The number of inhibitory effects induced by cPMv conditioning was much greater than the number of facilitatory effects (n=55; 73.3%). Inhibitory effects were more common across most ISIs, in particular with ISI15 (n=13) and ISI20 (n=13) and inhibition was also most powerful with these two ISIs (Figure 2.6E-F; dark gray bars). Although inhibition was predominant, it is worth noting that several facilitatory effects were also found with ISI5 and ISI10. In fact, in intrinsic hand muscles, facilitation of MEPs was more common than inhibition with ISI5 (facilitation n=5 and inhibition n=4).

The pattern of modulatory effects caused by cPMv conditioning in forearm muscles followed similar trends, although it tended to be even more inhibitory. Out of the significant modulation of MEPs, the proportion of facilitatory effects in forearm muscles was smaller than for intrinsic hand muscles (n=9; 9.6%) (Figure 2.6G). The highest number of facilitatory effects was evoked with ISI5 (n=4) and the magnitude of facilitation was also the greatest at this ISI (Figure 2.6H). No case of significant facilitation was found at ISI15 and ISI20. In sharp contrast, we found 85 cases (90.4%) in which conditioning of cPMv caused an inhibition of MEPs in forearm muscles, and inhibitory effects were much more common than facilitatory effects with all tested ISIs. The greatest numbers of inhibitory effects were induced with long delays between the C_{stim} and the T_{stim} (ISI15 n=17 and ISI20 n=15) or when the two stimuli were applied simultaneously (ISI0 n=16). The inhibitory effects of cPMv on MEPs in forearm muscles were also generally more powerful than facilitatory effects. The magnitude of inhibitory effects was comparable across ISIs, but inhibition was slightly more powerful with ISI15. Together these results support that cPMv is much more likely to have inhibitory than facilitatory effects on the outputs of M1 and that these inhibitory effects are more powerful. In contrast to iPMv, cPMv has comparable effects on M1 outputs to both intrinsic hand and forearm muscles.

Comparison of the general modulatory effects of iPMv and cPMv conditioning

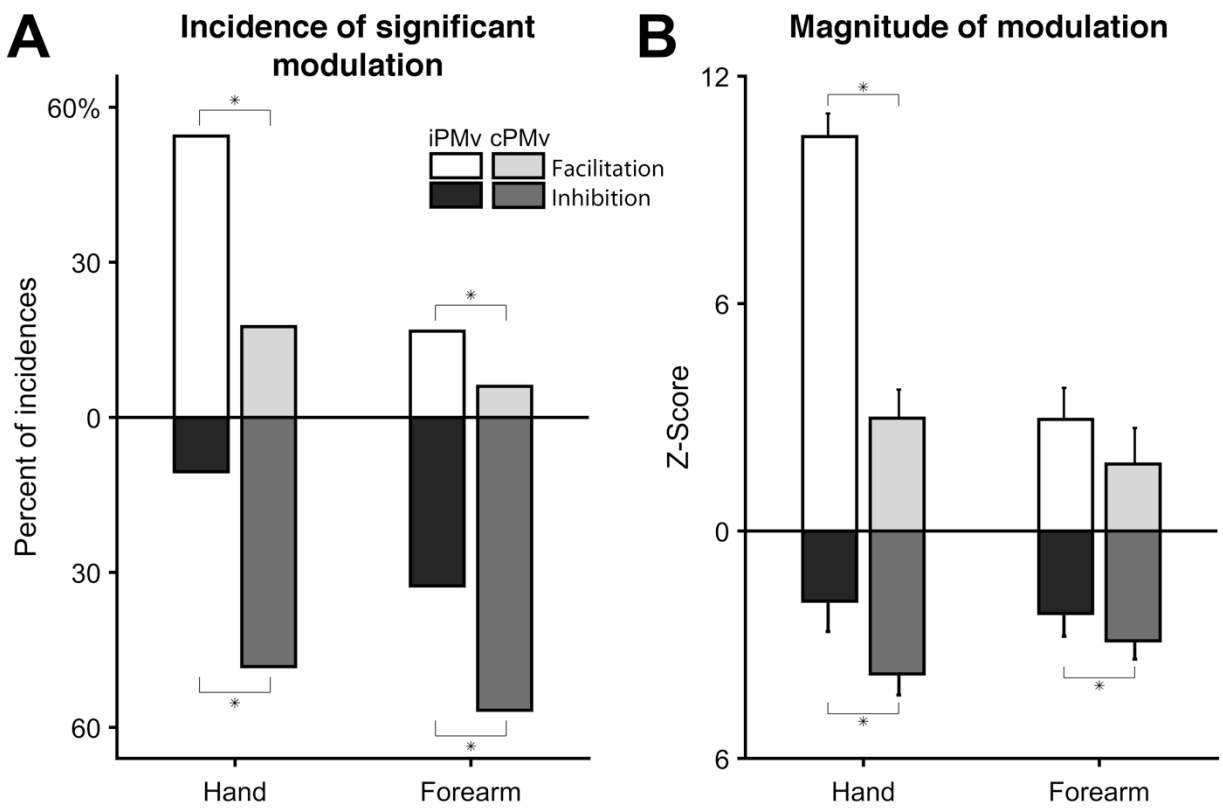

Figure 2.7 *General modulatory effects of iPMv and cPMv conditioning*

A) Incidence of facilitatory and inhibitory effects induced by iPMv and cPMv conditioning on the two muscle groups across all ISIs. The incidence of facilitation and inhibition was affected by the cortical location of the conditioning and the muscle group. For the intrinsic hand muscles (left bars), the number of facilitatory effects was greater when the conditioning was in iPMv (white; n=62; 54.4%) than in cPMv (light gray; n=20; 17.5%). Conversely, inhibitory effects were more common when the conditioning was delivered in cPMv (dark gray; n=55; 48.3%) than in iPMv (black; n=12; 10.5%). The modulatory effects on MEPs of forearm muscles followed a similar pattern (right bars). Facilitatory effects were more common when the conditioning stimulus was in iPMv (n=23; 16.7%) than in cPMv (n=9; 6.0%). In contrast, inhibitory effects were more common when the conditioning stimulus was in cPMv (n=85; 56.7%) than in iPMv (n=45; 32.6%). **B)** Magnitude of modulatory effects induced by iPMv and cPMv conditioning on the two muscle groups across all ISIs. For the intrinsic hand muscles (left bars), facilitation was much stronger with iPMv conditioning than with cPMv conditioning (mean Z-scores: iPMv=10.4; cPMv=3.0). In contrast, the magnitude of inhibitory effects in intrinsic hand muscles induced by conditioning of cPMv (mean Z-score=-3.8) was greater than iPMv (mean Z-score=-1.9). For forearm muscles, the

magnitude of facilitatory effects was comparable when the conditioning stimulus was in iPMv or cPMv (mean Z-scores: iPMv=2.9; cPMv=1.8). For inhibitory effects, conditioning of cPMv induced greater inhibitory effects (mean Z-score=-2.9) than iPMv (mean Z-score=-2.2). Asterisks show significant differences.

After combining all ISIs, we compared the incidence of facilitation and inhibition induced by iPMv and cPMv conditioning (Figure 2.7A). We used a chi-square test (X^2) followed by a post-hoc two-proportion Z-test. For intrinsic hand muscles, the distribution of modulatory effects produced by iPMv conditioning was different from that produced by cPMv (X^2=49.12; p<0.001). Conditioning of iPMv induced more facilitatory effects (54.4%) than cPMv (17.5%) (p<0.001), and cPMv induced more inhibitory effects (48.3%) than iPMv (10.5%) (p<0.001). Similar, although less pronounced effects were found for forearm muscles (X^2=19.52; p<0.001). The conditioning of iPMv induced more facilitatory effects (17.5%) than cPMv (6.0%) (p=0.004), and cPMd conditioning induced more inhibitory effects (56.7%) than iPMv (32.6%) (p<0.001).

We then compared the magnitude of the modulation produced by iPMv and cPMv conditioning (Figure 2.7B). A two-way ANOVA was used to compare the facilitatory effects and a second to compare the inhibitory effects using the location of the conditioning stimulation (iPMv or cPMv) and muscle group (intrinsic hand or forearm) as factors. The magnitude of facilitatory effects was strongly influenced by the location of the conditioning stimulus (F=9.68; p=0.002). However, arm muscles and hand muscles were not affected equally as indicated by a significant "location of conditioning x muscle group" interaction. Pairwise comparisons with Bonferroni correction revealed that the magnitude of the facilitatory effects induced with iPMv conditioning was significantly greater than with cPMv only for the intrinsic hand muscles (p<0.001). The magnitude of inhibitory effects was also significantly affected by the location of the conditioning stimulation (F=38.77, p<0.01). The inhibitory effects induced by cPMv were greater than those from iPMv for both intrinsic hand and forearm muscles. Thus, iPMv conditioning induced more facilitatory effects than cPMv for both intrinsic hand and forearm muscles, and the magnitude of the facilitatory effects in hand muscles induced by iPMv was greater than cPMv. In contrast, following cPMv conditioning there were more numerous and powerful inhibitory effects in both intrinsic hand and forearm muscles than after iPMv conditioning.

Categories of modulatory effects across ISIs induced by iPMv and cPMv conditioning

We analyzed how individual MEPs were modulated across ISIs and if there were differences between iPMv and cPMv conditioning (Figure 2.8A). To do so, we pooled together the MEPs from

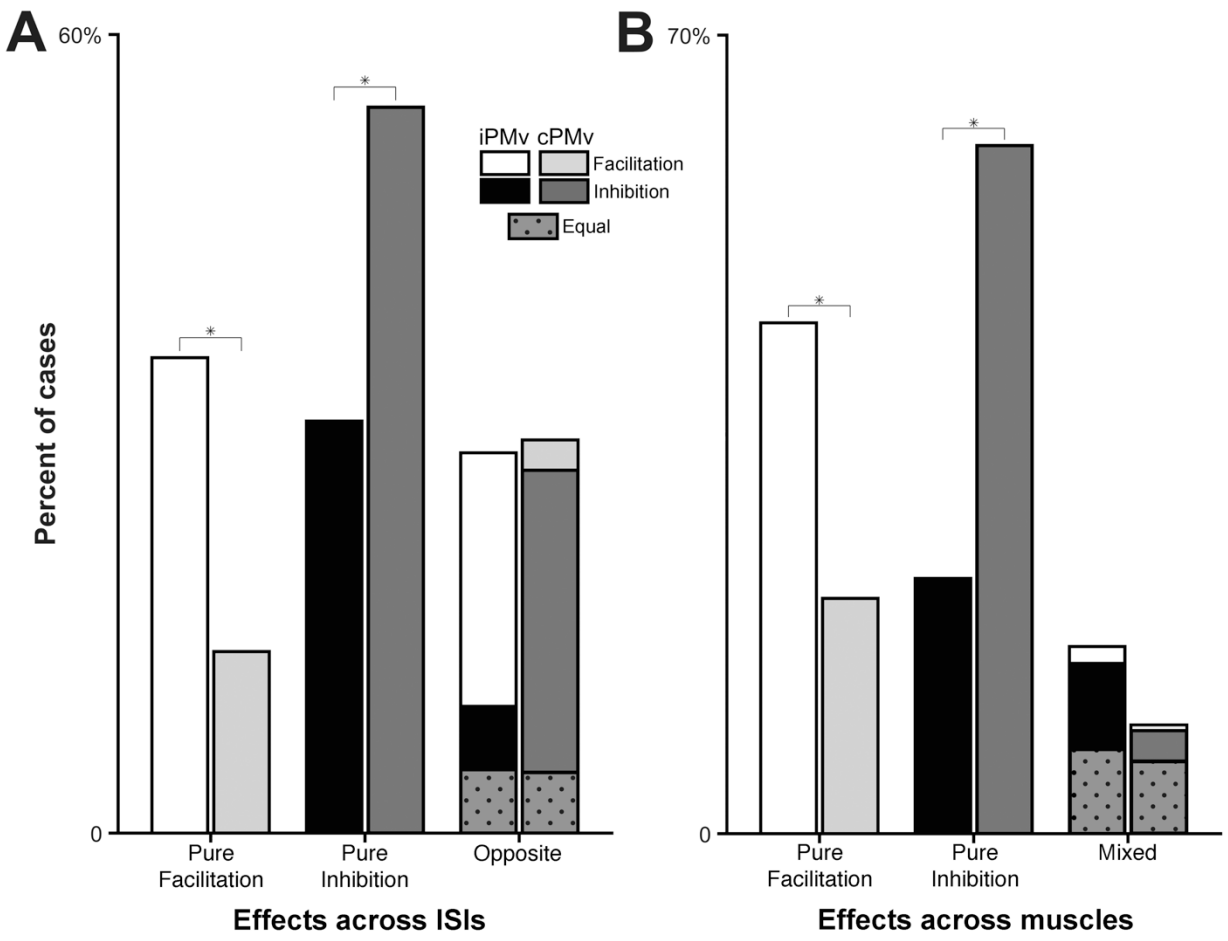

Figure 2.8 *Categories of modulatory effects from iPMv and cPMv across ISIs and recorded muscles*

A) Categories of conditioning effects across ISIs. Out of the 42 MEPs that were conditioned with iPMv stimulation, 15 MEPs were in Group Pure Facilitation (left, white bars) and 13 were in Group Pure Inhibition across ISIs (middle, black bars). In contrast, out of the 44 MEPs with cPMv conditioning, only 6 were in group Pure Facilitation (left, light gray bars) and 24 in Group Pure Inhibition (middle, dark gray bars). The count of MEPs in group Opposite was comparable after conditioning of both iPMv (n=12) and cPMv (n=13) (right bars). However, for MEPs conditioned by iPMv, facilitatory effects were more common across ISIs (right, white bars; n=8). In contrast, for MEPs conditioned by cPMv, inhibitory effects were more common across ISIs (right, dark gray bars; n=10; 76.9%). Dotted-gray bars on the right indicate the number of MEPs for which we found an equal number of occasions of inhibition or facilitation across ISIs. **B)** Summary of conditioning effects across muscles. There were more cases with Pure Facilitation across recorded muscles after

iPMv than cPMv conditioning (left bars). In contrast, there were more cases of Pure Inhibition across muscles after cPMv conditioning (middle bars). Finally, conditioning stimulation in both iPMv and cPMv induced comparable proportions of Mixed effects across muscles (i.e. simultaneous facilitation and inhibition in different muscles; right bars). Asterisks show significant differences.

the 6 muscles and counted the occurrences of facilitation and inhibition for each of the 6 tested ISIs. We found very few MEPs that were not modulated with any ISI (iPMv n=2; cPMv n=1). This supports that a very large proportion of M1 outputs to arm muscles can be modulated by iPMv (95.2%) and cPMv (97.7%) activation with the ISIs we tested. We classified the modulatory effects into 3 categories (Deffeyes et al., 2015). First, the conditioning of iPMv or cPMv could significantly facilitate the MEP with at least one ISI, but never significantly inhibit M1 outputs with any of the ISIs (Group Pure Facilitation). Second, the conditioning of iPMv or cPMv could significantly inhibit the MEP with at least one ISI, but never significantly facilitate M1 outputs with any of the ISIs (Group Pure Inhibition). Third, the conditioning of iPMv or cPMv could significantly facilitate the MEP with at least one ISI and also significantly inhibit the MEP with at least one ISI (Group Opposite).

We found that the source of the conditioning influenced the proportion of MEPs in each group (X^2=18.2; p<0.003). Post-hoc two-proportion Z-tests revealed that a greater proportion of MEPs modulated by iPMv conditioning were in Group Pure Facilitation (35.7%) in comparison to MEPs modulated by cPMv conditioning (13.6%) (p=0.02). In contrast, a greater proportion of MEPs modulated by cPMv conditioning were in Group Pure Inhibition (54.5%) in comparison to iPMv conditioning (31.0%) (p=0.03). Similar proportions of MEPs modulated by iPMv and cPMv were in Group Opposite (28.6% and 29.5% respectively) (p=0.90). However, very few of these MEPs were facilitated and inhibited with an equal number of ISIs (16.7% and 15.4% for iPMv and iPMv respectively). A larger proportion of MEPs conditioned by iPMv (66.6%) showed a predominance of facilitatory effects across ISIs and a much lower proportion showed a predominance of inhibitory effects (16.7%). For cPMv conditioning, a larger proportion of MEPs showed a predominance of inhibitory effects across ISIs (76.9%, versus 7.7% with a predominance of facilitatory effects). Altogether, these analyses show that pure facilitatory (Group Pure Facilitation) or predominantly facilitatory (Group Opposite) effects on MEPs across ISIs were much more common when the conditioning stimulus was applied in iPMv. In contrast, pure inhibitory (Group Pure Inhibition) or predominantly inhibitory (Group Opposite) effects on MEPs across ISIs were much more common when the conditioning stimulus was applied in cPMv.

Simultaneous modulation of recorded muscles with iPMv and cPMv conditioning

We also inspected the effects of iPMv and cPMv conditioning on the MEPs across muscles (Figure 2.8B). To do so, we pooled the MEPs with all 6 ISIs together and counted occurrences of facilitation and inhibition for each of 6 muscles. Since T_{stim} alone did not evoke any MEPs in the TB and only one in BB, these muscles were excluded from analyses. For both iPMv and cPMv protocols, we analyzed effects with each ISI separately (11 protocols x 6 ISIs = 66 total cases for iPMv and for cPMv). In one protocol with a given ISI, the conditioning of PMv could be only facilitatory on the MEPs of up to all 6 muscles (Group Pure Facilitation), could be only inhibitory on the MEPs (Group Pure Inhibition), or simultaneously facilitate and inhibit different combinations of muscles (Group Mixed) (Deffeyes et al., 2015). Out of the 66 cases with the conditioning stimulation in iPMv, we found 30 cases in Group Pure Facilitation (45.5%) and in 23 of these (34.8%), more than one muscle was simultaneously facilitated. Most often, however, MEPs in only 2 or 3 muscles were simultaneously facilitated. We found considerably fewer cases in Group Pure Inhibition (n=15; 22.7%) and in 8 of these, more than one muscle was simultaneously inhibited (12.1%). Finally, there were few cases in Group Mixed (n=11; 16.7%).

The profile of activation across muscles was quite different when the conditioning stimulation was in cPMd. There were many fewer cases in Group Pure Facilitation (n=13; 19.7%) and many more cases in Group Pure Inhibition (n=38; 57.6%). In 4 of the cases with Pure Facilitation (6.1%) and 29 of the cases with Pure Inhibition (43.9%), the effect was observed in more than one muscle simultaneously. Simultaneous inhibitory effects occurred most often in 4 or 5 muscles. As for iPMv, we found fewer cases in which simultaneous facilitation and inhibition were observed in the different muscles (Mixed; n=6; 9.0%). The number of effects in each category was significantly different if the conditioning was done in iPMv or cPMv (X^2=18.2; $p<0.001$). Post-hoc two-proportion Z-tests revealed that the incidence of cases of Pure Facilitation was greater after iPMv conditioning ($p=0.002$) and the proportion of Pure Inhibition was greater after cPMv conditioning ($p<0.001$). However, conditioning stimulation in both iPMv and cPMv induced comparable proportions of Mixed effects across muscles. Together, these results show that although both iPMv and cPMv can induce complex patterns of facilitation and inhibition across muscles, iPMv conditioning more frequently induces only facilitation and cPMv conditioning more frequently induces only inhibition across muscles of the hand and forearm.

We then wondered if simultaneous facilitation and inhibition of M1 outputs were specific to functional muscle groups. For example, if iPMv facilitates outputs to forearm flexor muscles, is it simultaneously inhibiting only outputs to forearm extensors or can it have opposite effects on any one muscle from which we recorded? For all the protocols that resulted in Mixed Effects in two or more muscles (Figure 2.8B; n=11 for iPMv and n=6 for cPMv), we counted the incidence of cases in which conditioning produced significant modulations in opposite directions for all 6 ISIs (i.e. significant facilitation in one muscle and significant inhibition in another). Figure 2.9 shows these results with the muscles divided into intrinsic hand (FPB; APB), forearm flexor (PL; FDS) and forearm extensor (EDC; ECU) categories.

When iPMv affected the MEP in a muscle (Figure 2.9A), it never simultaneously had opposite effects on the other muscle of the same category. Similarly, when iPMv affected the MEP in a forearm muscle, it rarely had opposite effects on MEPs in other forearm muscles, either flexors or extensors (≤6%). In contrast, when iPMv affected MEPs in intrinsic hand muscles, it could have opposite effect on MEPs in forearm muscles, and this was more common for forearm flexors (mean=27%) than extensors (mean=17%). Altogether, these data suggest that the simultaneous modulation of iPMv on M1 outputs is always in the same direction for muscles within the same functional group and very often has similar effects on forearm flexors and extensors. However, it can simultaneously have opposite effects on intrinsic hand and forearm muscles. In comparison to iPMv, when cPMv affected the MEP in a muscle there were fewer instances of opposite effects in other muscles (≤8% in all cases) (Figure 2.9B). Similar to iPMv, opposite effects of cPMv on the MEPs of muscles within the same category were quite uncommon. Opposite effects between intrinsic hand and forearm muscles were also infrequent (flexors, mean=6%; extensors, mean=5%). This suggests that cPMv conditioning typically affects M1 outputs to all muscles in the same direction, regardless of their function.

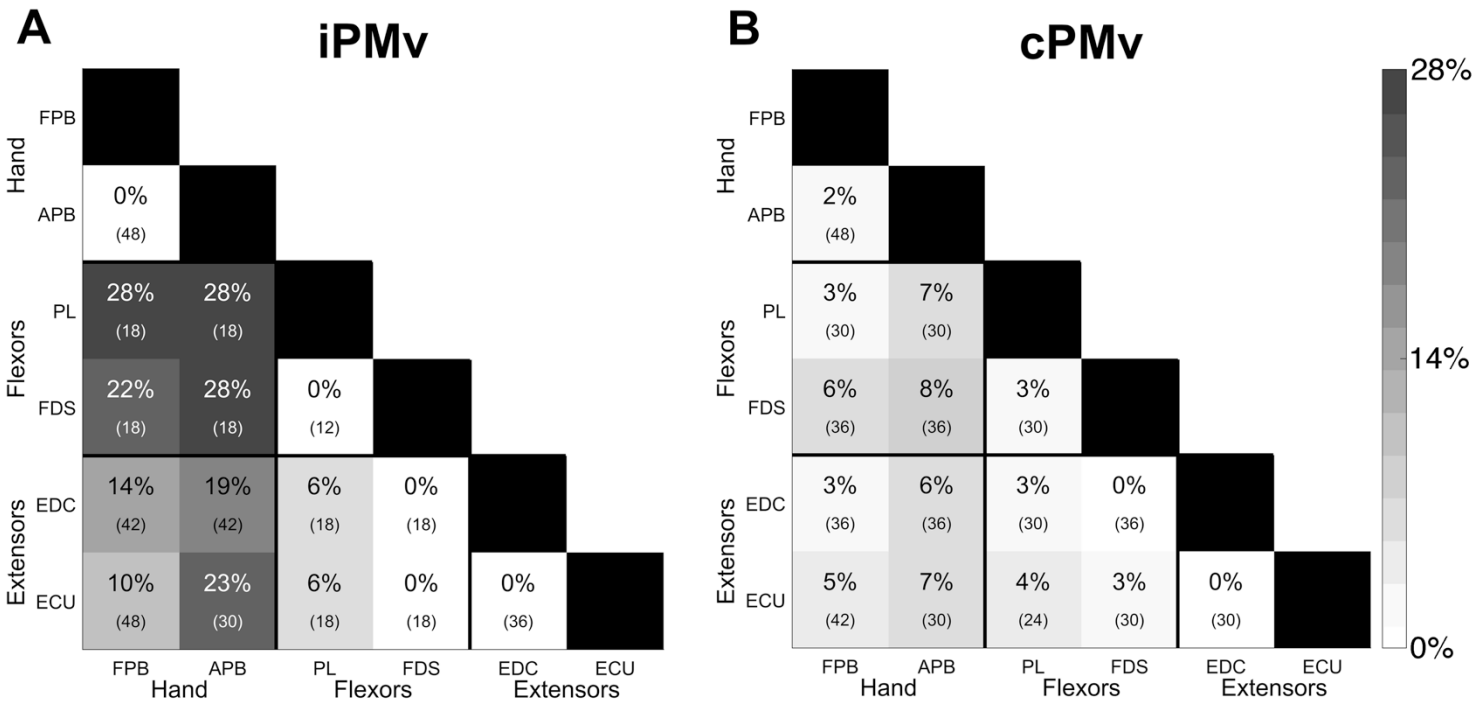

Figure 2.9 *Incidence of opposite effects of PMv conditioning across functional muscle categories*

Box diagram showing the incidence of simultaneous significant opposite effects across muscles (see Figure 2.8B; Mixed). In the box diagram, thick black vertical and horizontal lines separate muscles into functional categories (intrinsic hand, forearm flexors and forearm extensors). For each muscle (rows), we counted the number of cases in which the MEP was modulated in one direction (facilitatory or inhibitory) while the MEP in another muscle (columns) was modulated in the opposite direction. The percentage of opposite effects and the number of comparisons is indicated in parentheses in each box. The shade of gray for each box reflects the incidence of opposite effects. **A)** Incidence of opposite effects across muscles induced by iPMv conditioning. For example, we observed simultaneous MEPs in both FPB and APB in 48 cases (8 protocols X 6 ISIs), none of which were in the opposite direction (0%). The EDC and APB were simultaneously active in 42 cases (7 protocols X 6 ISIs). This time, in 8 of those cases EDC and APB were significantly modulated in opposite directions (19.0%). Overall, iPMv never had opposite effects on the other muscle of the same category and very rarely had opposite effects in forearm flexors and extensors. In contrast, there were a considerable number of cases in which iPMv had opposite effects on intrinsic hand and forearm muscles, and this was more common for forearm flexors than extensors. **B)** Incidence of opposite effects across muscles induced by cPMv conditioning. In comparison to

iPMv, there were fewer cases in which cPMv conditioning induced opposite effects in recorded muscles and there were no clear differences between muscle categories.

Discussion

Our objective was to study the influence of iPMv and cPMv on M1 outputs to intrinsic hand and forearm muscles in *Cebus apella* using paired-pulse protocols with ICMS techniques. We found that iPMv has predominantly facilitatory effects that are powerful. Facilitatory effects to intrinsic hand muscles were, however, much more common and stronger than to forearm muscles. The profile of modulation from cPMv was strikingly different. Conditioning stimulations in cPMv were much more often inhibitory. The inhibitory effects were stronger than facilitatory effects and the differences between intrinsic hand and forearm muscles were smaller than for iPMv. Nevertheless, the effects of both iPMv and cPMv were not homogeneous. Conditioning stimuli in iPMv could also inhibit, and those in cPMv could also facilitate M1 outputs. Our results provide new insights into the complex interactions occurring between PMv of the ipsi and contralateral hemisphere and M1. They show that iPMv and cPMv have very different patterns of modulatory effects on M1 outputs, predominantly facilitatory for iPMv and inhibitory for cPMv. The use of ICMS techniques, however, revealed complex neural populations within iPMv and cPMv, which may allow both these cortical areas to have facilitatory or inhibitory effects on M1 outputs that may be used depending on the requirements of the task.

The effect of iPMv on the outputs of M1 to intrinsic hand and forearm muscles

We found that stimulation of iPMv evoked mostly facilitatory effects on M1 outputs to the intrinsic hand muscles. The magnitude of facilitatory effects across the tested ISIs, with the most powerful effects evoked when the C_{stim} was delivered 10ms prior to the T_{stim}, is quite similar to that reported in sedated macaques (Cerri et al., 2003). However, in contrast to our findings, no inhibitory effects were reported in that study. In cebus monkeys, whereas inhibitory effects were much less frequent and less powerful than facilitatory effects, the conditioning of iPMv could also inhibit M1 outputs to intrinsic hand muscles. These inhibitory effects were induced with several ISIs, in particular when the C_{stim} in iPMv preceded the T_{stim} in M1 by 6ms or 10ms.

These results are more in line with reports in awake macaques performing a reach-to-grasp task (Prabhu et al., 2009). During reach, iPMv tends to facilitate M1 outputs when shorter ISIs are used (i.e. 0-1ms) and to be inhibitory with longer ISIs (5-6ms). In humans, studies using TMS have

also reported that iPMv conditioning can induce both facilitatory and inhibitory effects (Civardi et al., 2001; Munchau et al., 2002; Davare et al., 2008; Davare et al., 2009). It is therefore unlikely that the inhibitory effects from iPMv we found in cebus monkeys are due to interspecies differences. Rather, the wider range of modulatory effects may be explained by the higher number of cortical sites tested and the number of MEPs analyzed in comparison to previous studies in ketamine-sedated macaques (Cerri et al., 2003).

In contrast to intrinsic hand muscles, conditioning stimulations in iPMv induced many more inhibitory effects and less powerful facilitatory effects on forearm muscles. No studies have yet systematically compared effects of iPMv on MEPs in these different muscles. However, results from intracellular recordings of spinal motoneurons also suggest that iPMv affects intrinsic hand and forearm muscles differently (Shimazu et al., 2004). In these experiments, the conditioning of iPMv often induced a facilitation of the late excitatory post-synaptic potentials (EPSPs) evoked by M1 stimulations. The incidence of facilitatory effects was significantly greater in intrinsic hand motoneurons than in forearm flexor or extensor motoneurons.

It is not clear why the modulatory effects of iPMv on intrinsic hand and forearm muscles in cebus monkeys are so distinct. However, considering the magnitude of the discrepancies it is tempting to suggest that iPMv assumes different roles for the production of hand movements, depending on the function of the targeted muscle. Predominant and powerful facilitation of intrinsic hand muscles may allow iPMv to consolidate M1 outputs for the production of grasping forces required to squeeze objects. In contrast, the combination of facilitatory and inhibitory effects on M1 outputs to forearm muscles could be used to refine the coordination of simultaneous contractions of antagonist muscles necessary for the production of complex hand posture (Long et al., 1970; Brochier et al., 2004). One caveat that should be kept in mind is that the two intrinsic hand muscles recorded in the present study, like in many others, were from the thumb. It is not yet clear if iPMv has the same pattern of modulatory effects on other intrinsic hand muscles, for example the dorsal interosseous muscles, which have a very different impact on hand configuration.

The effects of cPMv on the outputs of M1 to intrinsic hand and forearm muscles

This is the first study to analyze the influence of cPMv on the outputs of M1. Conditioning stimulations in cPMv induced inhibitory effects much more often than facilitatory effects in both intrinsic hand and forearm muscles, and inhibition was most common with longer ISIs (15-20ms). This finding is in line with several studies demonstrating interhemispheric inhibition between other motor regions of the cortex. In cats, inhibitory responses in pyramidal tract neurons can be elicited from cortical sites spreading over a large territory in the contralateral M1 (Asanuma and Okuda, 1962). In contrast, facilitatory effects are only evoked with stimulation of a focal region homotopic to the recorded neuron. In humans, a number of paired-pulse TMS studies have also showed that M1 can exert robust inhibitory effects on its contralateral counterpart (Ferbert et al., 1992; Gerloff et al., 1998; Di Lazzaro et al., 2008). Although some studies have reported that interhemispheric facilitation can occur between the two M1s, these effects were weaker and only present under specific stimulation conditions (Ugawa et al., 1993; Hanajima et al., 2001).

The predominance of inhibitory effects across the hemispheres has also been suggested in several clinical and lesion studies. For example, small cortical lesions in one hemisphere in mice induce rapid increases of sensory evoked responses in the contralesional hemisphere (Mohajerani et al., 2011). Similarly in humans, there are many reports of increased cerebral blood flow and hyperexcitability in the contralesional hemisphere after stroke (Liepert et al., 2000; Marshall et al., 2000; Butefisch et al., 2003). Like what has been proposed for M1, inhibitory effects from cPMv may favor unilateral hand movements by restricting the outputs from the other hemisphere (Duque et al., 2005a; Grefkes et al., 2008; Reis et al., 2008). This could be of particular importance when skilled, precise, and often unilateral grasping movements are generated. The prevalent inhibitory effects of cPMv for both intrinsic hand and forearm muscles and with almost all ISIs suggest that this may be the primary role of interhemispheric interactions from the cPMv and that it occurs during several stages of the preparation and production of hand movements.

It is however worth noting that we also found many cases in which cPMv facilitated the outputs of M1. This was particularly common with ISIs of 5 and 10ms. In intrinsic hand muscles, facilitatory effects were even more numerous and more powerful than inhibitory effects with ISIs of 5ms. In humans, while the contralateral PMd has predominant inhibitory effects on M1 outputs

at rest (Mochizuki et al., 2004; Koch et al., 2007), it is mainly facilitatory in the early stage of movement preparation (Liuzzi et al., 2010; Liuzzi et al., 2011). This early facilitation appears to favor the coordination of independent, anti-phase, movements of the two hands. It is also possible that the facilitatory effects from cPMv are predominant in the early phases of movement preparation, something that should be tested in awake monkeys or humans.

The greater facilitatory effects to intrinsic hand muscles with mid-latencies of ISIs we tested (5-10ms) highlight another potential role of cPMv more closely related to movement production. Again for PMd, the interhemispheric modulatory effects were also shown to change during the production of movements. For example, whereas the left PMd has inhibitory effects on the right M1 at rest, it becomes facilitatory during voluntary movements of the left hand (Bestmann et al., 2008). Facilitatory effects from premotor areas of the hemisphere ipsilateral to the moving limb may be specifically used in more complex and challenging tasks. In this context, instead of exerting interhemispheric inhibition to prevent undesirable movements, these premotor areas could play a more active role in the production of outputs to the moving hand (Horenstein et al., 2009). Alternatively, facilitatory effects from cPMv may be of particular use to coordinate bilateral contraction of distal muscles.

Effects of iPMv and cPMv conditioning across tested ISIs

For both iPMv and cPMv, we found cases where the conditioning stimulation only facilitated M1 outputs, only inhibited or could both facilitate and inhibit M1 outputs across tested ISIs. These results support that there are small populations of neurons within iPMv and cPMv that systematically either facilitate or inhibit the outputs of M1 to a given muscle, even if more or less time is given for the conditioning stimulus to affect different neural pathways. Perhaps these populations can be used when outputs to a given muscle must be strictly inhibitory or facilitatory, independently of the stage of movement preparation or production. This could be the case when a finite hand position is intended and produced for a specific grasp.

In both iPMv and cPMv, we also found a comparable number of cases that could have both facilitatory and inhibitory effects on the same muscles, depending on the timing of the conditioning

stimulus. These changes of effects across ISIs could be due to the pathway taken by the conditioning stimulus to exert its effect on M1 output. For example, some stimulated neurons in iPMv may have direct facilitatory connections onto M1 pyramidal neurons and yet, other nearby iPMv neurons excite GABAergic interneurons that then contact onto the same M1 pyramidal neurons (Ghosh and Porter, 1988). Such complex patterns of modulation may help with the rapid phasic contractions of muscles when changes of hand configurations is the intended goal, as required by skillful dexterous manipulation of objects.

Effects of iPMv and cPMv conditioning across recorded muscles

Stimulus-triggered averaging of EMG studies in primates have shown that any given M1 site generally has consistent effects, either only facilitatory or only inhibitory, on the arm and hand muscles in its field (Kasser and Cheney, 1985; McKiernan et al., 1998). However, simultaneous facilitation and inhibition of different muscles can also occasionally be observed. With paired-pulse stimulations, similarly we found that both iPMv and cPMv most often had consistent effects across the muscle field targeted by the M1 outputs.

In both iPMv and cPMv we also found cases with mixed effects within the muscle field of the M1 site. A closer look at the muscles in which these opposite effects occurred also suggests a more specific pattern of modulation from iPMv than cPMv. The incidence of simultaneous modulation of MEPs in opposite directions following cPMv conditioning was comparable for the different categories of muscles (intrinsic hand, forearm flexor or forearm extensor). In contrast, the conditioning of iPMv induced many more opposite effects on M1 outputs to intrinsic hand and forearm muscles. This suggests a potentially different modulatory role of iPMv for these two muscle groups. Perhaps when the final hand configuration is obtained, iPMv favors powerful facilitation of M1 outputs to intrinsic hand muscles to exert the grasping forces while limiting the modulation of M1 outputs to the forearm muscles that only need to maintain the hand's posture.

Chapitre 3 - Contrasting modulatory effects from the dorsal and ventral premotor cortex on primary motor cortex outputs

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Abstract

The dorsal and ventral premotor cortices (PMd and PMv) each take part in unique aspects for the planning and execution of hand movements. These premotor areas are components of complex anatomical networks that include the primary motor cortex (M1) of the two hemispheres. One way PMd and PMv could play distinct roles for hand movements is by differently modulating the outputs of M1. Yet, patterns of effects from PMd and PMv on the outputs of M1 have not been systematically compared. Our goals were to study how PMd within the same (i.e. ipsilateral or iPMd) and in the opposite hemisphere (i.e. contralateral or cPMd) can shape M1 outputs and compare these effects to those induced by PMv. We used paired-pulse protocols with intracortical microstimulation techniques in sedated cebus monkeys while recording electromyographic (EMG) signals from intrinsic hand and forearm muscles. A conditioning stimulus was delivered in iPMd or cPMd concurrently or prior to a test stimulus in M1. The patterns of modulatory effects from PMd were compared to those from PMv collected in the same animals. Striking differences were revealed. Conditioning stimulation in iPMd induced more frequent and powerful inhibitory effects on M1 outputs in comparison to iPMv. In the opposite hemisphere, cPMd conditioning induced more frequent and powerful facilitatory effects than cPMv. These contrasting patterns of modulatory effects could allow PMd and PMv to play distinct functions for the control of hand movements and predispose them to undertake different, perhaps somewhat opposite roles in motor recovery after brain injury.

Introduction

The dorsal premotor cortex (PMd) is an area of the frontal lobe involved in the planning and execution of forelimb movements. Microstimulation studies in monkeys have shown the existence of a distal forelimb representation within PMd from which movements of the forearm, wrist and fingers can be evoked (Preuss et al., 1996; Raos et al., 2003; Dea et al., 2016). Neurons in this cortical region discharge during the preparatory and execution phases of hand movements (Wise, 1985; Kurata and Tanji, 1986; Riehle and Requin, 1989) and their pattern of activity can be tuned to specific types of grasps (Raos et al., 2004).

PMd is not only involved in the control of the contralateral hand but also participates in the preparation and production of bilateral and ipsilateral movements. For example, both neural recording studies in monkeys (Kermadi et al., 2000) and imaging studies in humans (Meyer-Lindenberg et al., 2002) have shown that activity in PMd can increase during complex bimanual hand movements. Moreover, the activity of many neurons in PMd is modulated when preparing and performing tasks with either hand (Tanji et al., 1988; Kermadi et al., 2000). Finally, human imaging studies also revealed that hemodynamic activity in PMd can increase as a function of task complexity when performing ipsilateral sequential finger movements (Sadato et al., 1996).

In addition to PMd, primates have several other premotor areas, each sending effective outputs to the motoneurons of forelimb and hand muscles (He et al., 1993; Dum and Strick, 2002; Boudrias et al., 2010a). While these premotor areas are all part of the cortical motor network, they each have a unique pattern of connections (Dum and Strick, 2005; Dea et al., 2016; Hamadjida et al., 2016; Kaas and Stepniewska, 2016) and appear to undertake some specialized functions for the control of hand movements. For example, while PMd seems more involved in intersegmental coupling, arm trajectory and geometry for arm and hand movements, PMv is primarily concerned with pre-shaping the hand to accurately match the properties of the objects to be grasped (Kurata, 1993; Scott et al., 1997; Rizzolatti and Luppino, 2001; Davare et al., 2006).

One way PMd and PMv can participate in the production of hand movements is by modulating the outputs of the primary motor cortex (M1). To date, several human studies have investigated the modulatory effects of PMd and PMv on the outputs of M1 using transcranial magnetic stimulation (TMS). They have shown that PMd and PMv within the same hemisphere (i.e. ipsilateral or iPMd and iPMv) and in the opposite hemisphere (i.e. contralateral or cPMd and

cPMv) can have a wide range of effects on M1 outputs (Civardi et al., 2001; Koch et al., 2006; O'Shea et al., 2007; Davare et al., 2008; Prabhu et al., 2009; Buch et al., 2010; Groppa et al., 2012; Quessy et al., 2016). One largely unexplored question is how the pattern of modulatory effects of PMd on M1 outputs compares to that of PMv. Different patterns of modulations from the two areas may provide a means for them to assume their unique roles in the preparation and production of hand movements. They could also predispose premotor areas to undertake distinct functions and have different impacts on the large-scale reorganization of ipsilesional and interhemispheric network after brain injury (Grefkes and Fink, 2012; Silasi and Murphy, 2014).

To address some of these questions, we investigated the modulatory effects of iPMd and cPMd on M1 outputs using paired-pulse protocols with intracortical microstimulation (ICMS) methods in sedated cebus monkeys. In these protocols, a conditioning pulse (C_{stim}) was delivered in either iPMd or cPMd simultaneously or prior to a test pulse (T_{stim}) in M1 with different interstimulation intervals (ISIs). Modulatory effects were quantified in electromyographic (EMG) signals from forearm and intrinsic hand muscles. We then compared the modulatory effects of PMd with those from PMv collected in the same animals (Quessy et al., 2016).

Methods

Subjects

Four adult female capuchin monkeys (*Cebus Apella;* CB1 (1.9kg), CB2 (1.3kg), CB3 (1.4kg) and CB4 (1.2kg)) were used in this study. Monkeys were group housed and supplied with food and water *ad libitum*. The experimental protocol followed the guidelines of the Canadian Council on Animal Care and was approved by the Comité de Déontologie de l'Expérimentation sur les Animaux (CDEA) of the Université de Montréal.

Surgical procedures

All procedures were performed in a terminal experiment. Details of surgical procedures were described previously (Quessy et al., 2016). Anesthesia was induced with 15 mg/kg of ketamine hydrochloride and transitioned to ~2-3% isoflurane (Furane; Baxter, Deerfield, IL, USA) in 100% oxygen. The animal received Dexamethasone 2 (Vetoquinol®; 0.5 mg/kg) and Mannitol 20% (1,500 mg/kg) to prevent inflammation and swelling of the brain. To maintain proper hydration, lactated ringer's solution (10 ml/kg/h) was continuously injected intravenously. Body temperature was kept near 36.5°C throughout the procedures and blood oxygen saturation and heart rate were continuously monitored.

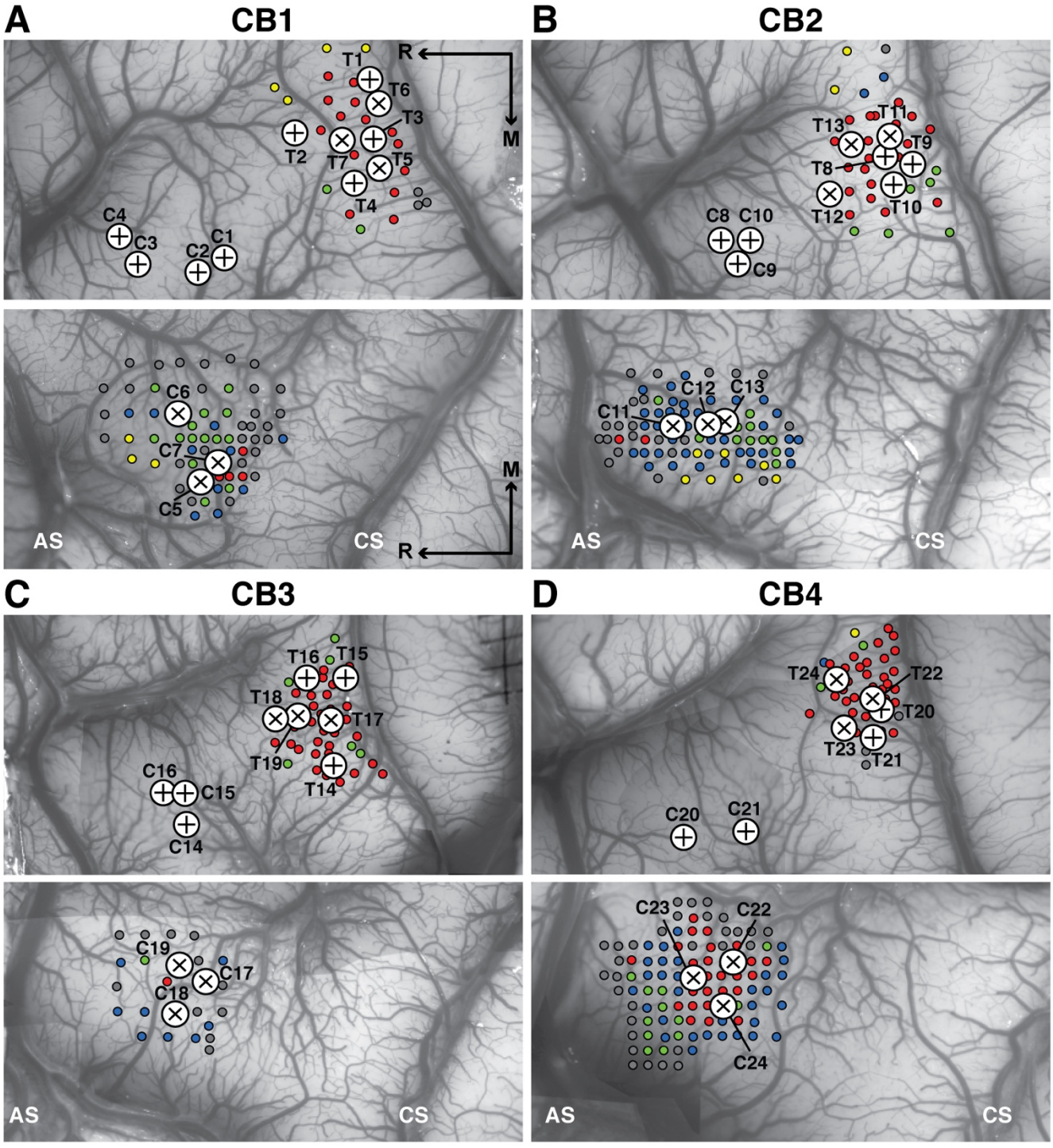
Insulated, multistranded microwires (Cooner Wire, Chatsworth, CA, USA) were implanted intramuscularly for the recording of electromyographic (EMG) signals. In CB1, 6 muscles in both arms were implanted (*flexor pollicis brevis* (FPB)*, extensor carpi ulnaris* (ECU)*, extensor digitorum communis* (EDC)*, palmaris longus* (PL)*, biceps brachii* (BB) and *triceps brachii* (TB). In the other 3 monkeys, in addition to these 6 muscles, the *abductor pollicis brevis* (APB) and the *flexor digitorum superficialis* (FDS) were also implanted. The accurate placement of EMG wires was confirmed with stimulation of each muscle through the implanted wires and observation of the evoked movements. After EMG microwires implantation, craniotomies and durectomies were performed to expose M1 and iPMd in one hemisphere as well as cPMd in the opposite hemisphere.

Paired-pulse stimulation and EMG recording

After the surgical procedures, gas anesthesia was turned off and the animal was kept deeply sedated with intravenous injections of ketamine (~10 mg/kg/10 minutes) and Diazepam (Valium; 0.01mg/kg/hr) for electrophysiological data collection. To facilitate the identification of stimulation sites related to hand movements for the paired-pulse protocols, we first located the hand representations in M1 and premotor areas using standard ICMS trains (13 monophasic cathodal pulses of 0.2ms delivered at 350Hz) delivered at 1Hz (Deffeyes et al., 2015; Dea et al., 2016; Hamadjida et al., 2016; Quessy et al., 2016). Within these identified hand representations (Figure 3.1), we then searched for stimulation sites in layer V (~1800μm) to place the electrode for the C_{stim} in either iPMd or cPMd and the electrode for the T_{stim} in M1 with two independent micromanipulators. At each cortical site tested, we visually inspected EMG signals on an oscilloscope to confirm that ICMS trains evoked clear EMG responses in at least one contralateral intrinsic hand or forearm muscle. Only such cortical sites were kept for the paired-pulse protocols. Thus, all cortical stimulation sites selected in this study were located in clearly identified distal forelimb representations, as defined with ICMS trains.

Once the electrodes were in place, stimulations were switched from trains to single pulses. Both the C_{stim} and T_{stim} were cathodal single square pulses of 0.2ms duration delivered through single wire insulated tungsten electrodes (FHC Bowdoin, ME USA). The stimulation intensities for the C_{stim} and T_{stim} were established online, based on evoked EMG activity in the arm contralateral to each electrode. If EMG activity was evoked in multiple muscles in the contralateral arm, the muscle with the lowest threshold (current at which EMG activity was evoked by ~50% of single pulses) was chosen to establish the current intensity. The current intensity for the C_{stim} was set at 75% of the EMG threshold (range=38-225 μA, mean=167 μA). If no EMG activity was evoked with up to 300μA with single pulses, the current intensity of the C_{stim} was set to 225 μA. The current intensity for the T_{stim} was typically set to 125% of threshold (range=50-300 μA, mean=163 μA). However, if the evoked activity was too small or too big with this intensity value, it was adjusted to a level producing clear, submaximal responses. This insured that the motor evoked potential (MEP) evoked by the T_{stim} could be either increased or decreased by the C_{stim} at all cortical sites tested.

After the establishment of the stimulation intensities, a paired-pulse stimulation protocol was initiated. Within each protocol, stimulations could be delivered through the conditioning


A
CB1
R
M
T1
T6
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T2
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C4
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C5
M
R
AS
CS
B
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T11
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C18
AS
CS
D
CB4
T24
T22
T20
T23
T21
C20
C21
C23
C22
C24
AS
CS
Distal forelimb representation
Digits
Wrist and Forearm
iPMd protocols
cPMd protocols
Elbow and Shoulder
Face
No response
R
M
M
R
5mm

Figure 3.1 *Cortical locations of the Cstim and Tstim electrodes selected for paired-pulse protocols*

Motor mapping data evoked with ICMS trains (small colored dots) and cortical locations selected for C_{stim} and T_{stim} electrodes (large circles) for the paired-pulse protocols in CB1 **(A)**, CB2 **(B)**, CB3 **(C)** and CB4 **(D)**. In each monkey, we first located the hand representations of M1 and cPMd with motor mapping techniques using ICMS trains and visual inspection of evoked movements. The hand representation of iPMd was then easily located by stimulating cortical sites in the cortical area homotopic to cPMd in the ipsilateral hemisphere. Evoked movements with ICMS trains at threshold current intensity are color-coded according to the legend at the bottom of the figure. Within M1, iPMd and cPMd, only cortical sites evoking clear EMG activity in at least one intrinsic hand or forearm muscle with ICMS trains were selected for paired-pulse protocols. All of these sites were thus in the distal forelimb representations of M1, iPMd and cPMd. Large circles with + show cortical sites used in protocols testing the effects of iPMd on M1 outputs and large circles with x show cortical sites used in protocols testing the effects of cPMd. CS: central sulcus; AS: arcuate sulcus; M: medial; R: rostral.

electrode only (C-only trials), the test electrode only (T-only trials), or through both electrodes (paired stimulations or paired-pulse trials; C+T) using one of 6 different interstimulation intervals (ISIs). For iPMd conditioning, the paired stimulations were presented simultaneously (ISI0) or with the C_{stim} preceding the T_{stim} by 1ms (ISI1), 2ms (ISI2), 4ms (ISI4), 6ms (ISI6) or 10ms (ISI10). For cPMd conditioning, the paired stimulations were presented simultaneously (ISI0) or with the C_{stim} preceding the T_{stim} by 2.5ms (ISI2.5), 5ms (ISI5), 10ms (ISI10), 15ms (ISI15) or 20ms (ISI20).

Using several ISIs allows the outputs of neurons stimulated by the C_{stim} and the T_{stim} electrodes to interact through the various pathways they share and provides information about the temporal specificity of modulatory effects from the conditioned area. When comparing the modulatory effects of diverse cortical areas, here PMd and PMv, these detailed patterns of interaction can highlight specific latencies with which the two areas exert their most similar or divergent effects on the outputs of M1.

In the present set of experiments, we opted to test ISIs around the time windows of the fast cortico-cortical effects between iPMd or cPMd (C_{stim}) and M1 (T_{stim}). For the ipsilateral hemisphere, short latency intrahemispheric conduction time between premotor areas and M1 is estimated to ~1-2ms (Godschalk et al., 1984; Tokuno and Nambu, 2000). Accordingly, it can be proposed that ISI1 and ISI2 are more likely to favor direct projections from iPMd onto output producing neurons in M1 (e.g. corticospinal) (Ghosh and Porter, 1988; Tokuno and Nambu, 2000). In contrast, simultaneous stimulation of iPMd and M1 (ISI0) could favor downstream convergent projections along the neuraxis, for example in the spinal cord (He et al., 1993). However, summation of the C_{stim} effects onto cortically mediated I-waves in M1 could also explain the effects with ISI0, ISI1 and ISI2 (Shimazu et al., 2004; Maier et al., 2013). Finally, ISI4, ISI6 and ISI10 may favor effects carried by slower conducting fibers, oligosynaptic projection pathways from PMd to M1 or give time for the C_{stim} to induce changes of excitability at downstream sites of convergence with M1 outputs.

For the contralateral hemisphere, as short latency interhemispheric conduction time between motor areas is estimated to ~2-6ms (Asanuma and Okuda, 1962; Matsunami and Hamada, 1984), ISI2.5 and ISI5 can be proposed to favor direct projections onto M1 output neurons. Similar pathways as the ones suggested above for the ipsilateral effects could be favored with shorter (e.g.

downstream convergence with ISI0) and longer ISIs (e.g. oligosynaptic pathways with ISI10, ISI15 and ISI20).

Nevertheless, it should be kept in mind that the strength of testing several ISIs is to provide information about the range of possible modulatory effects of the conditioned area on the outputs of the tested area. Since stimulations are delivered at the cortical level and the modulatory effects are identified at the level of muscles through EMG recordings, the locus of interactions is uncertain.

For each of the 8 stimulation conditions (C-only, T-only and C+T with 6 ISIs), a total of 150 trials were collected (total number of trials per protocol=1,200). In CB1 and CB2, data for each condition was collected in three blocks of 50 trials delivered at 3Hz and the order of the blocks was randomized across conditions (Deffeyes et al., 2015). In CB3 and CB4, the condition used for each trial was randomly selected until a total of 150 trials delivered at 3Hz was collected for each condition (Quessy et al., 2016). This latter design was an improvement of our custom written acquisition software. Nevertheless, we confirmed that the EMG responses acquired from both designs were stable throughout data collection by comparing the responses obtained with the T-only trials from the first 75 trials to those obtained with the last 75 trials using two-sample t-tests (CB1 and CB2: $t=-1.57$; $p=0.12$; CB3 and CB4: $t=-0.73$; $p=0.48$). This supports that the randomization of blocks of trials was sufficient to prevent potential effects that could result from the serial acquisition of data from different conditions (see also (Deffeyes et al., 2015; Quessy et al., 2016)).

Following data collection for a protocol, the two electrodes were relocated to different cortical positions and another protocol was initiated. These procedures were repeated until a total of 2 to 4 cortical sites were tested for iPMd and cPMd in each animal. For iPMd conditioning, EMG activity was concurrently recorded from 6 muscles for 4 protocols in CB1 and 8 muscles for 8 protocols in the other 3 monkeys. We thus collected 88 EMG signals under 8 conditions (total of 704 MEPs). For cPMd conditioning, EMG activity was concurrently recorded from 6 muscles for 3 protocols in CB1 and 8 muscles for 9 protocols in the other 3 monkeys. Accordingly, we collected 90 EMG signals under 8 conditions (total of 720 MEPs).

A RZ5 real-time processor (Tucker Davis Technologies (TDT), Alachua, FL, USA) with a custom software was used to conduct paired-pulse stimulation protocols and record EMG data. One component of the custom software controlled the stimulations generated by an IZ2 stimulator

(Tucker Davis Technologies (TDT), Alachua, FL, USA) while another component controlled the data acquisition. EMG signals from each channel were recorded at 4.9 kHz. Raw EMG data were stored for offline analysis.

Electromyographic (EMG) data analysis

EMG data were analyzed offline with custom written MatLab (Version R2014a; Nantick, MA, USA) code. The continuously recorded raw EMG signals were separated into individual trials and aligned on the end of the last stimulation (i.e. the C_{stim} for C-only trials and the T_{stim} for the T-only and the 6 paired-pulse trials). Then, the EMG responses were analyzed in a window of 30ms after the end of the stimulation (Figure 3.2A-C). The raw EMG was full-wave rectified and smoothed using a 5 points moving average (window=1.02ms).

We first established if the T_{stim} alone (T-only trials) in M1 induced a detectable MEP and that this response was large enough to distinguish either increases or decreases of activity potentially induced in paired stimulations trials (C+T) (Quessy et al., 2016). All T-only trials (n=150) for each muscle were averaged and the MEP was compared to the baseline activity recorded in a window of 30ms prior to the stimulus onset. If the average MEP peak amplitude resulting from the T-only trials was greater than 3 standard deviations (SD) above the average baseline, it was considered significant and kept for subsequent analyses. While the C_{stim} intensity was set to 75% of threshold value, we also verified the absence of responses offline. Across the entire data set, we found and discarded 4 cases in which the average response induced with the C-only trials was greater than 3SD above the average baseline and in which the presence of a potential MEP was confirmed with visual inspection.

To study the modulation of MEPs peak amplitude by the C_{stim} delivered in iPMd or cPMd, we compared the amplitude of the evoked response in paired-pulse trials to a probability distribution of predicted peak amplitudes based on the combination of responses in C-only and T-only trials (Figure 3.2D-E). This process has been described in detail previously (Quessy et al., 2016). First, to produce predicted traces, we linearly summed all possible combinations (n=22,500) of single C-only traces (n=150) with single T-only traces (n=150). Out of this population of

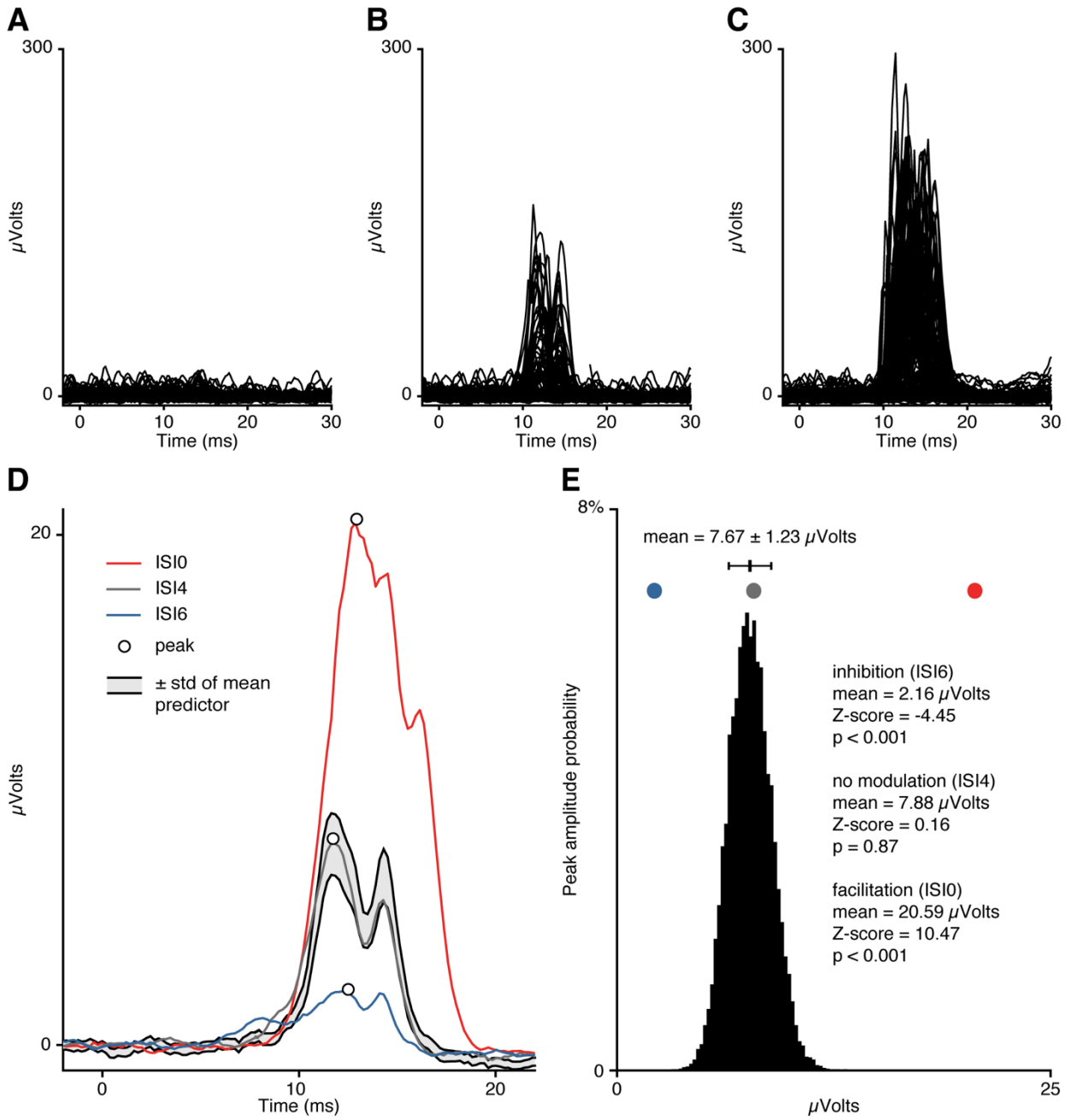

Figure 3.2 *Comparison of the conditioned responses with the probability distribution*

A) Example of responses evoked in the *palmaris longus* (PL) with the C-only condition (n=150) in iPMd for a given protocol. As current intensity for C_{stim} was subthreshold, no clear MEP is observed. Data across all following panels were recorded in PL during the same protocol. **B)** Responses evoked with the T-only condition (n=150) and **C)** when the C_{stim} and T_{stim} were delivered simultaneously (ISI0; n=150). **D)** Example of mean responses evoked in PL in the C+T condition with different ISIs in relation with the ± standard deviation (gray area) obtained from the

predicted MEPs (see methods). Here traces show responses when the C_{stim} preceded the T_{stim} by 0ms, 4ms and 6ms (ISI0 (red), ISI4 (gray) and ISI6 (blue)). Open circles show EMG peak maximum values. **E)** Histogram of the probability distribution of predicted MEP peak amplitudes (n=10,000). The histogram shows the probability of occurrence (y axis) of predicted peaks with different amplitudes (x axis). The black line and whiskers above the histogram indicate the mean and standard deviation of the probability distribution. The colored dots on top show the values of the average peak amplitude obtained with ISI0 (red), ISI4 (gray) and ISI6 (blue) from the traces in D. The average peak amplitude with ISI0 was clearly greater than the probability distribution (Z-score=10.47; $p<0.001$) and the effect of iPMd was considered significantly facilitatory with this ISI. In contrast, the average peak amplitude with ISI6 was smaller than the probability distribution (Z-score=-4.45; $p<0.001$) and the effect of iPMd was considered significantly inhibitory with this ISI. Finally, the average peak amplitude with ISI4 was within the probability distribution (Z-score=0.16; $p=0.87$) and it was concluded that iPMd had no effect on M1 outputs to PL with this ISI.

predicted traces, 150 trials were randomly selected and averaged to generate an average predicted MEP. The peak amplitude of the predicted MEP was calculated (peak maximum-peak minimum voltage value) within a 30ms window after the end of the stimulus. The random selection of 150 trials to generate an average predicted MEP and the calculation of its peak amplitude was repeated 10,000 times to produce a probability distribution of predicted peak amplitudes (Figure 3.2E). The average peak amplitude of the MEPs obtained with the paired-pulse trials (C+T) with each ISI (n=150 per ISI) was compared to the probability distribution to determine the direction of modulation (facilitation, inhibition or no modulation; Figure 3.2E). The normalized strength of the modulatory effects of PMd on M1 outputs was obtained by calculating the Z-score of the average MEP peak amplitude of C+T trials with each ISI. The modulation of M1 outputs by PMd conditioning was considered significant when the Z-score of the average MEP peak amplitude of C+T trials with a given ISI differed by more than 1.96SD from the mean of the distribution of predicted peak amplitudes ($p \leq 0.05$).

While there is potential for non-linearity when performing summation of rectified EMG signals (Baker and Lemon, 1995), this issue was minimized in our experiments by the use of sub-threshold stimulus intensity for the C_{stim} and inclusion of only large responses evoked by the T-only trials (>3SD; average SD above baseline=63.48 ± 43.63). Moreover, in paired-pulse trials our assessment of the incidence of facilitation and inhibition was based on significant modulations (± 1.96SD). Errors caused by non-linearity are expected to be small (Baker et al., 1998) and are thus likely to have been excluded from these analyses.

Results

We conducted a total of 24 paired-pulse protocols in 4 cebus monkeys in order to study the modulatory effects of iPMd (n=12) and cPMd (n=12) on M1 outputs (see Figure 3.1). As described above, clear EMG activity in at least one digit or forearm muscle was evoked with ICMS trains from all cortical sites selected for the C_{stim} and T_{stim} electrodes. Hence, this study specifically focuses on the interactions between outputs from the distal forelimb representations in iPMd, cPMd and M1.

For all 24 protocols, stimulations with the T_{stim} electrode (T-only trials) evoked a significant MEP (see methods) in at least one and up to 7 muscles of the contralateral arm (total=81 MEPs). Similar to previous reports in awake monkeys (Lemon et al., 1987; Baker et al., 1998), we observed that single-pulse ICMS in M1 commonly evoked an early facilitation followed by a longer-lasting suppression (~60% of cases) in unrectified EMG signals. However, because baseline EMG signals values were close to zero in sedated preparations, the late suppression effects were not apparent in rectified signals. Significant MEP were more common in the FPB (n=23), APB (n=16), ECU (n=14) and EDC (n=12) and less common in PL (n=7) and FDS (n=6). Because we specifically positioned our T_{stim} electrode at cortical sites that evoked EMG activity in digit or forearm muscles, as expected, we found very few MEPs in proximal arm muscles (BB=3, TB=0). We thus only analyzed MEPs in intrinsic hand (FPB and APB; total n=39 MEPs) and forearm muscles (ECU, EDC, PL and FDS; total n=39 MEPs).

Figure 3.3 is an intensity plot that provides a complete view of the effects of iPMd (Figure 3.3A) and cPMd (Figure 3.3B) conditioning on the MEPs in intrinsic hand and forearm muscles (total of 37 MEPs modulated by iPMd and 41 MEPs modulated by cPMd). For iPMd, we found a total of 18 MEPs in intrinsic hand muscles collected from 11 protocols (4 cortical sites with only one MEP in either FPB or APB and 7 cases with simultaneous MEPs in FPB and APB) and a total of 19 MEPs in forearm muscles collected from 9 protocols (4 cortical sites with an MEP in only one of the forearm muscles). For cPMd, we found a total of 21 MEPs in intrinsic hand muscles collected from 12 protocols (3 cases with only one MEP in either FPB or APB and 9 cases with simultaneous MEPs in FPB and APB) and a total of 20 MEPs in forearm muscles collected from 8 protocols (3 cases with only one MEP in one of the forearm muscles). Each line in the left column

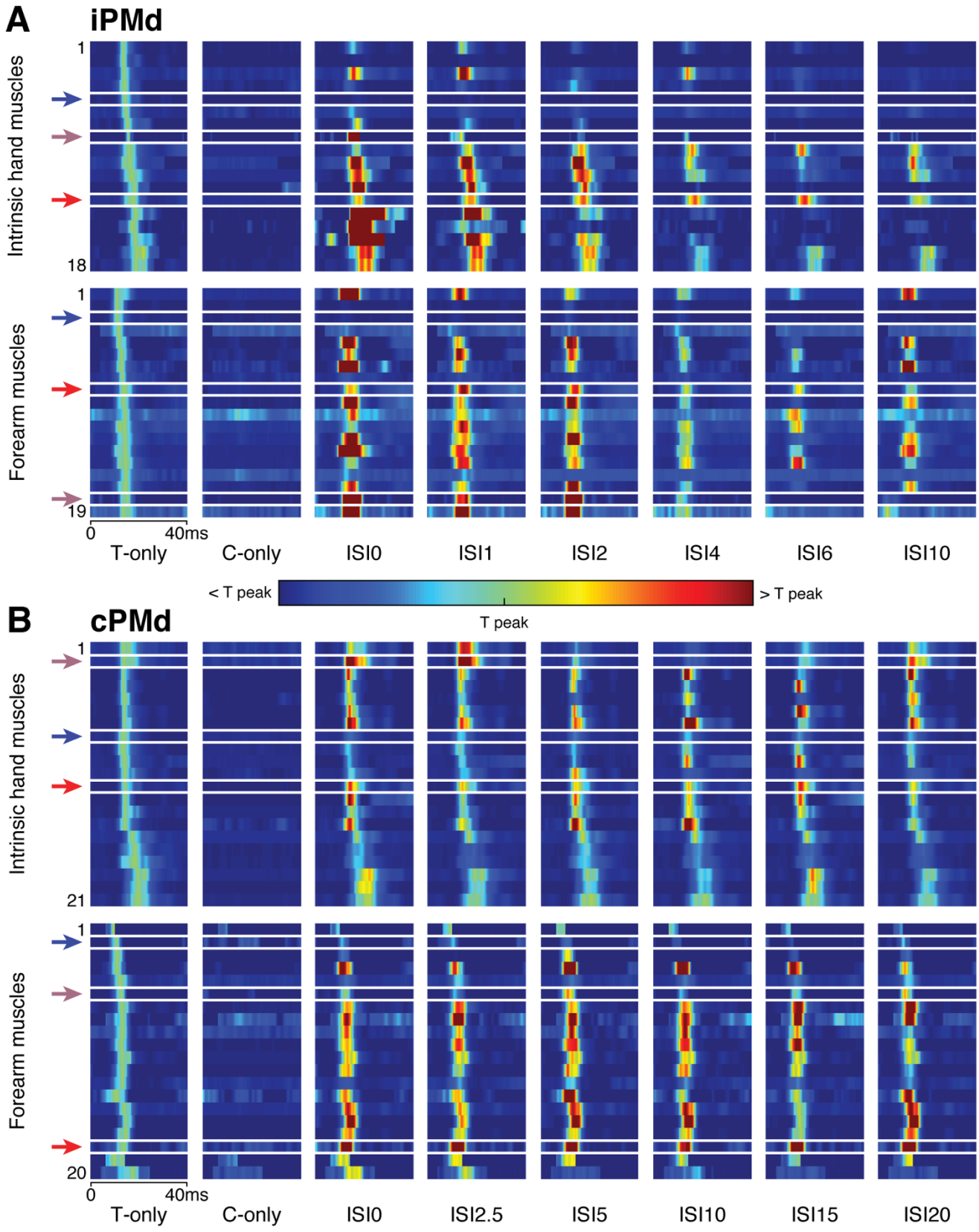
A
iPMd
Intrinsic hand muscles
Forearm muscles
1
18
19
0
40ms
T-only
C-only
ISI0
ISI1
ISI2
ISI4
ISI6
ISI10
< T peak
T peak
> T peak
B
cPMd
Intrinsic hand muscles
Forearm muscles
1
21
20
0
40ms
T-only
C-only
ISI0
ISI2.5
ISI5
ISI10
ISI15
ISI20

Figure 3.3 *Complete data set of modulatory effects of iPMd and cPMd conditioning on M1 outputs*

A) Effects of iPMd conditioning on the 18 MEPs recorded in intrinsic hand muscles (FPB, APB; upper panel) and the 19 MEPs recorded in forearm muscles (ECU, EDC, PL, FDS; lower panel). Columns from left to right show the activity for 40ms after the stimulation (time=0) evoked in T-only trials, C-only trials and the 6 different tested ISIs (0, 1, 2, 4, 6 and 10ms). These responses are normalized to MEP peak intensity in the T-only condition (color scale below). Each row in the plots is an individual MEP, ordered from top to bottom based on the peak latency in the T-only condition. Because the intensity of the C_{stim} was purposefully subthreshold, little activity is observed in the C-only condition. The red, blue and purple arrows respectively highlight examples in which the conditioning stimulation in iPMd induced pure facilitation, pure inhibition and opposite effects across ISIs. In general, iPMd conditioning appeared to induce more cases of facilitation (yellow to red colors) with shorter ISIs (ISI0, ISI1 and ISI2) and more cases of inhibition (light to dark blue colors) with longer ISIs (ISI4, ISI6 and ISI10). **B)** Effects of cPMd conditioning on the 21 MEPs recorded in intrinsic hand muscles (upper panel) and the 20 MEPs recorded in forearm muscles (lower panel). Columns and arrows are as in A, although different latencies were used for the tested ISIs (0, 2.5, 5, 10, 15 and 20ms). In general, cPMd appeared to induce more facilitation with all ISIs, especially in forearm muscles.

shows the MEP evoked with the T_{stim} only. The color intensity in the other columns is normalized to the peak value of this MEP and presents the responses with the C_{stim} only and the paired stimulations conditions (C+T) with the different ISIs. In paired-pulse conditions, the conditioning stimulations induced a wide range of modulatory effects on M1 outputs across the different ISIs and this was the case in intrinsic hand and forearm muscles. In both muscle groups, we found cases in which the conditioning stimulus in iPMd or cPMd could increase the peak amplitude of the MEP (facilitation) or decrease it (inhibition) with any of the tested ISIs. In some cases, when the conditioning stimulation had an effect on the MEP, it was always facilitatory, regardless of the ISI (pure facilitation across ISIs; see red arrows in Figure 3.3). In other cases, when the conditioning stimulation had an effect, it was always inhibitory, regardless of the ISI (pure inhibition across ISIs; see blue arrows in Figure 3.3). Finally, the conditioning stimulation could facilitate the MEP with some ISIs and inhibit the MEP with others (opposite effects across ISIs; see purple arrows in Figure 3.3).

In spite of this variability, some notable general trends in the data set were also visible. Conditioning stimulations in iPMd appeared to be more likely to facilitate M1 outputs with shorter ISIs and this was the case for both intrinsic hand and forearm muscles (Figure 3.3A). With longer ISIs, inhibitory effects became much more frequent in both muscle groups. In comparison, conditioning stimulations in cPMd generally seemed to induce more facilitatory effects and modulatory effects from cPMd appeared to be less affected by ISIs (Figure 3.3B), and these trends were more obvious in forearm than intrinsic hand muscles.

Quantification of the modulatory effects of iPMd on M1 outputs with each ISI

For each significant MEP in T-only trials, we created a probability distribution of predicted peak amplitudes based on the combination of responses in C-only and T-only trials (see methods and Figure 3.2). We then compared the MEPs in paired stimulations conditions to this distribution to identify significant modulatory effects. For each tested ISI, we counted the number of significant facilitatory and inhibitory effects and the average magnitude of these two types of modulation with each ISI (Figure 3.4).

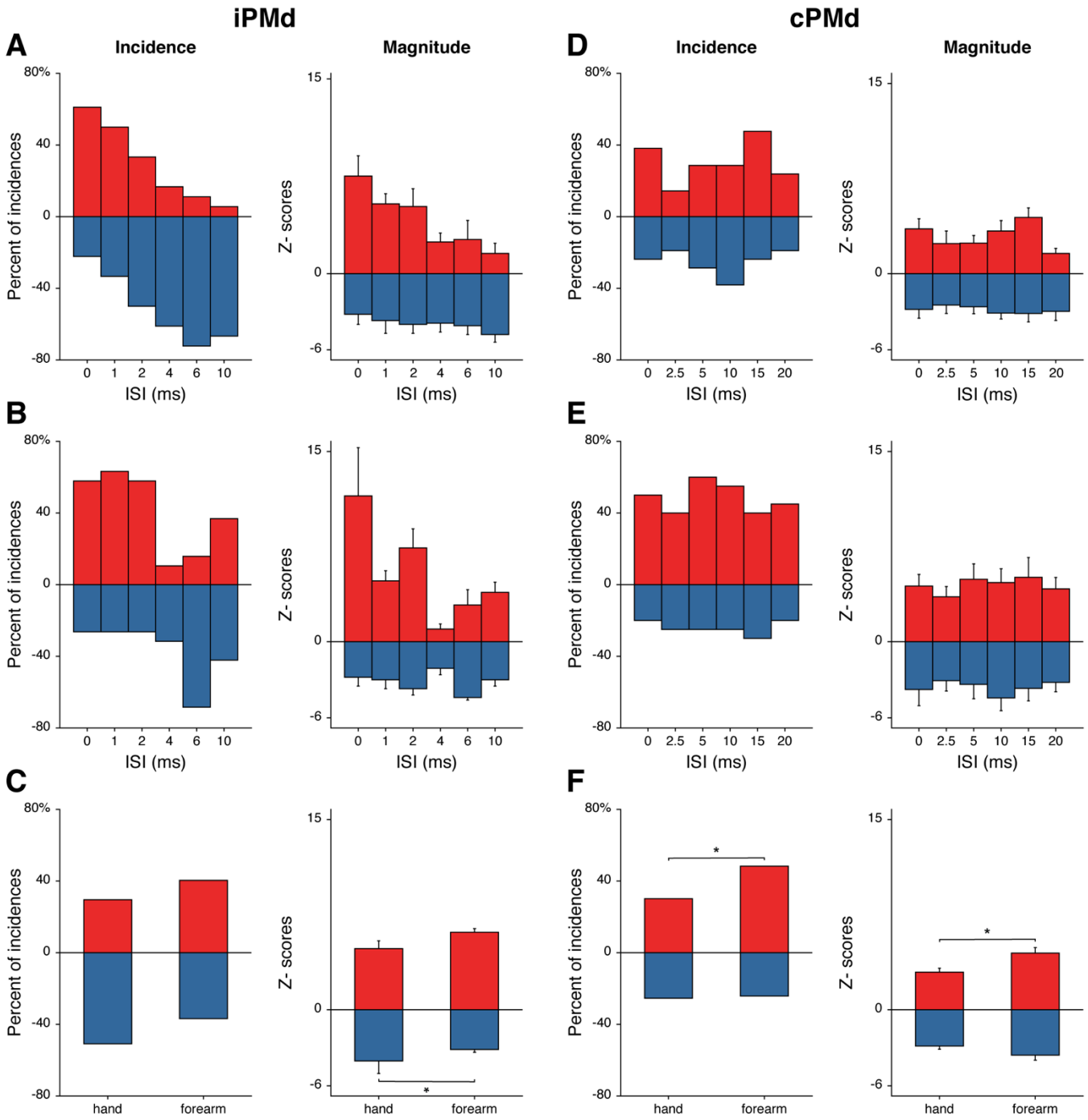

Figure 3.4 *Quantification of modulatory effects of iPMd and cPMd with each ISI*

A) Incidence (left panel) and magnitude (right panel) of modulations in intrinsic hand muscles produced by iPMd with the different tested ISIs. In the left panel, each bar shows the proportion of the 18 MEPs that were significantly facilitated (red) or inhibited (blue) with each ISI. For example, when both the C_{stim} and T_{stim} were applied simultaneously (ISI0), 11 of the 18 MEPs (61.1%) had

a significant increase of peak amplitude in comparison to the distribution of predicted peaks (facilitation) and 4 (22.2%) had a significant decrease of peak amplitude (inhibition). The right panel shows the magnitude of the modulations in intrinsic hand muscles produced by iPMd conditioning. The histogram presents the mean (± standard errors; SE) of the positive and negative Z-scores with the different ISIs. **B)** Incidence (left panel) and magnitude (right panel) of modulations in forearm muscles produced by iPMd conditioning with each ISI. **C)** Data with all tested ISIs pooled for the incidence (left panel) and magnitude (right panel) to reveal the general modulatory effects produced by iPMd conditioning. Inhibitory effects induced by iPMd were significantly more powerful in intrinsic hand than in forearm muscles. **D)** Incidence (left panel) and magnitude (right panel) of modulations in intrinsic hand muscles produced by cPMd with the different tested ISIs. **E)** Incidence (left panel) and magnitude (right panel) of modulations in forearm muscles produced by cPMd with each ISI. **F)** Data with all tested ISIs pooled for the incidence (left panel) and magnitude (right panel) of modulations produced by cPMd conditioning. Facilitatory effects were significantly more common and more powerful in forearm than in intrinsic hand muscles. * Significant effects.

For intrinsic hand muscles, out of the 108 MEPs (18 significant responses with T-only conditioned with 6 ISIs), we found 87 cases in which iPMd conditioning significantly modulated M1 outputs (80.6%). Out of these significant effects, we found fewer cases of facilitation (n=32; 36.8%) than cases of inhibition (n=55; 63.2%). Figure 3.4A (left panel) shows the incidence of significant facilitation and inhibition with each tested ISI. With each ISI, we found some cases in which iPMd conditioning induced significant facilitation and some cases in which it induced significant inhibition. However, in this figure it becomes obvious that facilitatory effects were much more common when the C_{stim} preceded the T_{stim} by shorter ISIs (ISI0 n=11; ISI1 n=9 and ISI2 n=6) and less common with longer ISIs (ISI4 n=3; ISI6 n=2 and ISI10 n=1). In contrast, inhibitory effects were less likely to occur with shorter ISIs (ISI0 n=4 and ISI1 n=6) and more likely to occur with longer ISIs (ISI4 n=11; ISI6 n=13 and ISI10 n=12).

We also studied the magnitude of the modulation of M1 outputs to intrinsic hand muscles produced by iPMd conditioning using the relative measure of the intensity of the modulatory effect (Z-score; see methods) (Figure 3.4A, right panel). These analyses revealed that the magnitude of the facilitatory and inhibitory effects with each ISI generally followed a similar pattern as the one described for incidence. Facilitatory effects were more powerful with shorter ISIs, especially with ISI0. Although the impact of ISIs on the magnitude of inhibitory effects was much milder, there was a tendency for inhibitory effects to be more powerful with longer ISIs. Thus, iPMd induced more frequent and powerful facilitatory effects with shorter ISIs and induced more frequent and powerful inhibitory effects with longer ISIs. It is worth noting that one cortical site in iPMd (C14 in CB3; see Figure 3.1) generated particularly strong facilitatory effects in intrinsic hand muscles with ISI0 (> 20 times greater than those of other cortical sites). Thus, we decided to remove data from this site in our analyses and figures showing the magnitude of modulatory effects from iPMd. Including it would dramatically increase the magnitude of facilitatory effect with ISI0, without impacting the rest of the pattern of facilitatory effects for each ISI.

We then performed the same analyses for MEPs in forearm muscles. Out of the 114 studied MEPs (19 significant responses with T-only conditioned with 6 ISIs), we found 88 cases (77.2%) in which peak amplitude of the MEP was significantly greater or smaller than the probability distribution. Out of these significant effects, we found comparable proportion of facilitation (n=46; 52.3%) and inhibition (n=42; 47.7%). In general, the pattern of modulation in forearm muscles

followed similar trends as the ones described for intrinsic hand muscles. Conditioning stimulations in iPMd induced more facilitation with shorter ISIs (ISI0 n=11; ISI1 n=12 and ISI2 n=11) and more inhibition with longer ISIs (ISI6 n=13 and ISI10 n=8) (Figure 3.4B, left panel). For the magnitude of effects (Figure 3.4B, right panel), we found that facilitatory effects were more powerful with shorter ISIs, especially with ISI0 and less powerful with longer ISIs. Although the magnitude of inhibitory effects tended to be of comparable strength for each ISI, they were least powerful with ISI4.

We pooled data from all tested ISIs to compare the modulations of iPMd on intrinsic hand and forearm muscles using a chi-square test (X^2) (Figure 3.4C, left panel). We found that the distribution of modulatory effects influencing intrinsic hand muscles was not different from that influencing forearm muscles (X^2=4.63; p=0.10). We also compared the magnitude of the modulatory effects induced by iPMd on intrinsic hand and forearm muscles using two-sample t-tests (Figure 3.4C, right panel). One two-sample t-test was used to compare the facilitatory effects and a second to compare inhibitory effects. We found that the magnitude of facilitatory effects was not significantly different (t=-1.07; p=0.29). However, the magnitude of inhibitory effects was greater in intrinsic hand than in forearm muscles (t=-2.45; p=0.02).

Quantification of the modulatory effects of cPMd on M1 outputs with each ISI

For intrinsic hand muscles, out of the 126 MEPs (21 significant responses with T-only conditioned with 6 ISIs), we found 70 cases in which cPMd conditioning significantly modulated M1 outputs (55.6%). Out of these significant effects, we found 38 cases of facilitation (54.3%) and 32 cases of inhibition (45.7%). Hence, in contrast to iPMd, cPMd induced a greater proportion of facilitatory than inhibitory effects on intrinsic hand muscles. Figure 3.4D (left panel) shows the incidence of significant facilitation and inhibition with each ISI we tested. The most common facilitatory effects were found when the C_{stim} preceded the T_{stim} by 15ms (ISI15 n=10) and when the two stimulations were delivered simultaneously (ISI0 n=8). Facilitatory effects were least common with ISI2.5 (n=3). With all tested ISIs, we also found cases in which cPMd conditioning induced significant inhibitory effects. Inhibitory effects were most likely to occur when the C_{stim} preceded the T_{stim} by 10ms (ISI10 n=8).

The magnitude of modulatory effects induced by cPMd conditioning on intrinsic hand muscles was not affected much by the ISIs. However, facilitation was strongest with ISI15 and ISI0 (Figure 3.4D, right panel). Thus with these two ISIs, facilitatory effects were not only most frequent (see Figure 3.4D, left panel), they were also stronger. The magnitude of the inhibitory effects was even more stable with the various ISIs tested, supporting that when present, inhibitory effects in intrinsic hand muscles were of relatively comparable strength, regardless of the delay between the C_{stim} and T_{stim}.

In forearm muscles, out of the 120 studied MEPs (20 significant responses with T-only conditioned with 6 ISIs), we found 87 cases (72.5%) in which peak amplitude of the MEP was significantly greater or smaller than the probability distribution. Again in contrast to iPMd, many more of these significant effects were facilitatory (n=58; 66.7%) in comparison to inhibitory (n= 29; 33.3%). Following cPMd conditioning, we found a greater proportion of facilitatory than inhibitory effects in forearm muscles with each tested ISI (Figure 3.4E, left panel). Facilitatory effects were slightly more common when the C_{stim} preceded the T_{stim} with mid-range ISIs (ISI5 n=12 and ISI10 n=11) and inhibitory effects were quite stable across ISIs. For the magnitude of modulatory effects of cPMd conditioning on MEPs in forearm muscles (Figure 3.4E, right panel), we found that ISIs did not affect responses much.

We compared the incidence of effects induced by cPMd on intrinsic hand and forearm muscles using a chi-square test (X^2) and found that the distribution of modulatory effects influencing intrinsic hand muscles was different from that influencing forearm muscles (X^2=10.1; p=0.01). A post-hoc two-proportion Z-test confirmed that conditioning of cPMd induced significantly more facilitatory effects in forearm (48.3%) than in intrinsic hand muscles (30.2%) (p=0.003). In contrast, the incidence of inhibitory effects was not significantly different between intrinsic hand and forearm muscles (p=0.82). We also compared the magnitude of the modulatory effects induced by cPMd on intrinsic hand and forearm muscles using two-sample t-tests (Figure 3.4F, right panel). One two-sample t-test was used to compare facilitatory effects and a second to compare inhibitory effects. We found that while the magnitude of facilitatory effects was greater in forearm than intrinsic hand muscles (t=-2.74; p=0.007), the magnitude of inhibitory effects of the two muscle groups was not significantly different (t=1.60; p=0.11).

Comparison of the pattern of modulatory effects of iPMd and iPMv with each ISI

In a previous study, we analyzed the effects evoked by PMv conditioning on the outputs of M1 (Quessy et al., 2016). Because these data were collected in the same animals as the ones analyzed in the present study for PMd, this allows for direct comparison of the modulatory effects from the two premotor areas. Furthermore, the distribution of significant MEPs evoked with T-only trials across recorded muscles was comparable for iPMd and iPMv (X^2=0.50; p=1.55) and for cPMd and cPMv (X^2=1.5; p=0.96).

Figure 3.5A (left panel) compares the number of significant modulatory effects evoked by iPMd and iPMv conditioning in all muscles combined with each tested ISI. With shorter ISIs (ISI0, ISI1 and ISI2), iPMd conditioning induced a greater proportion of facilitatory effects compared to iPMv. With longer ISIs (ISI4, ISI6 and ISI10) iPMd induced a smaller proportion of facilitatory effects. In contrast, inhibitory effects were more frequently induced by iPMd conditioning with all ISIs tested, and this difference increased with longer ISIs. Figure 3.5A (right panel) compares the magnitude of modulatory effects induced by iPMd and iPMv with each tested ISI. Note that these analyses also excluded site C14 from CB3 (see above). Still, facilitatory effects from iPMd were stronger than those from iPMv with ISI0. They were weaker with all other ISIs, in particular ISI10. In contrast, iPMd induced more powerful inhibitory effects than iPMv with all tested ISIs.

Figure 3.5B combines data obtained with all tested ISIs for iPMd and iPMv. To compare the incidence of modulatory effects (Figure 3.5B, left panel), we used a chi-square test (X^2) followed by a post-hoc two-proportion Z-test. We found that the distribution of modulatory effects produced by iPMd conditioning was different from that produced by iPMv (X^2=34.2; p<0.001). Although iPMd and iPMv conditioning induced similar proportions of facilitatory effects (35.1% and 33.7%, respectively) (p=0.75), iPMd induced significantly more inhibitory effects (43.7%) than iPMv (22.6%) (p<0.001). To compare the magnitude of modulatory effects of iPMd and iPMv (Figure 3.5B, right panel), two-sample t-tests were used. One two-sample t-test comparing the magnitude of facilitatory effects showed no significant difference between iPMd and iPMv conditioning (mean Z-scores=5.50 and 7.52, respectively; t=1.52; p=0.13). Another two-sample t-test comparing the magnitude of inhibitory effects showed that iPMd induced significantly greater inhibitory effects than iPMv conditioning (mean Z-scores=-3.60 and -2.08, respectively; t=6.72;

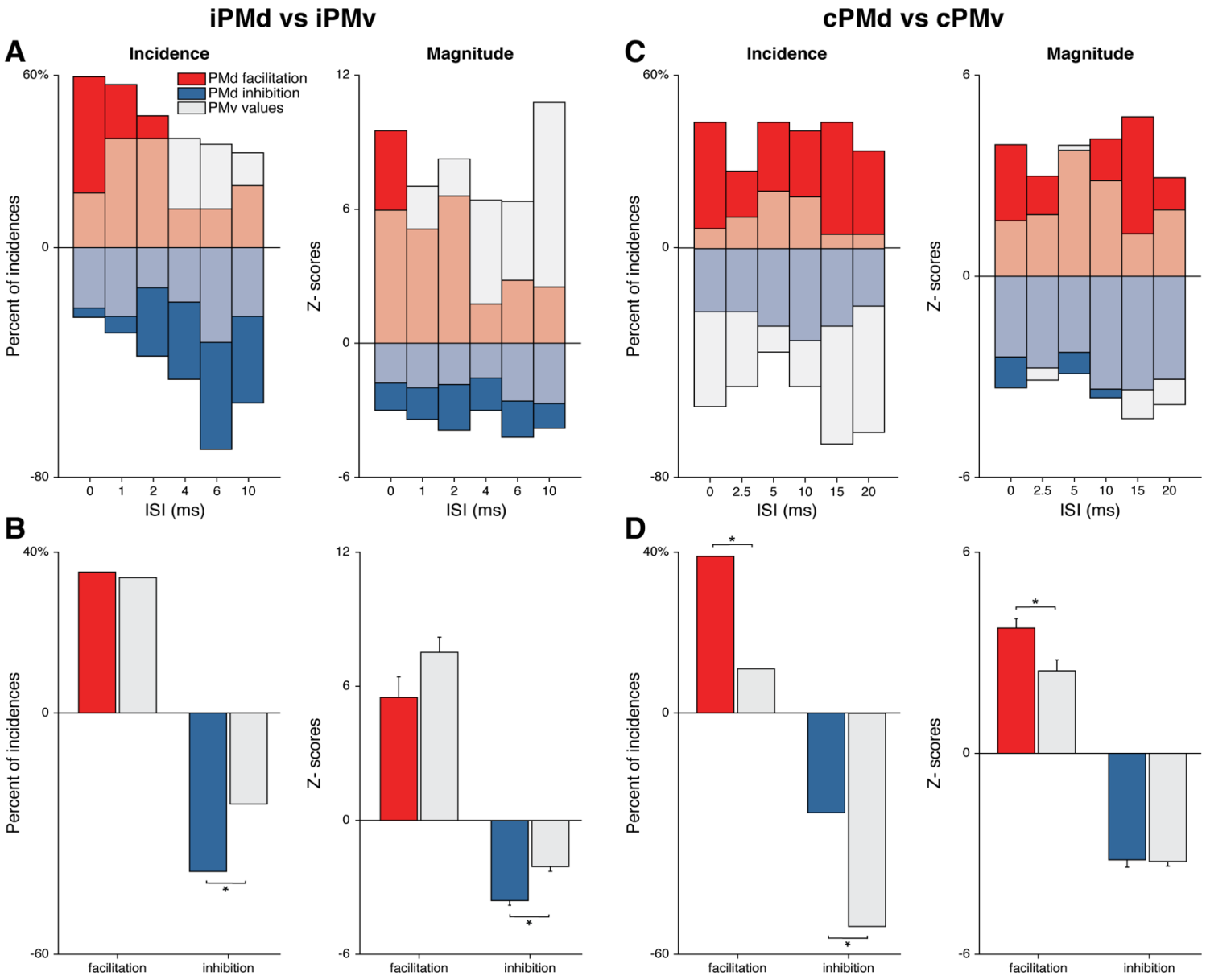

Figure 3.5 *Comparison of the modulatory effects of PMd and PMv with each ISI*

Comparison of the incidence (left panel) and magnitude (right panel) of modulations produced by iPMd and iPMv conditioning in all muscles combined with each ISI. The effects induced with iPMv conditioning (see-through gray) are overlaid on those induced with iPMd (red=facilitation and blue=inhibition). Accordingly, when PMv data overlap PMd data the bar appears in light grayish red for facilitation and in light grayish blue for inhibition. **B)** Data with all tested ISIs pooled for the incidence (left panel) and magnitude (right panel) of modulations produced by iPMd and iPMv. Inhibitory effects were significantly more frequent and more powerful following iPMd

compared to iPMv conditioning. **C)** Comparison of the incidence (left panel) and magnitude (right panel) of modulations produced by cPMd and cPMv conditioning in all muscles combined with each ISI. **D)** Data with all tested ISIs pooled for the incidence (left panel) and magnitude (right panel) of modulations produced by cPMd and cPMv. Facilitatory effects were significantly more frequent and more powerful after cPMd compared to cPMv conditioning. Inhibitory effects were significantly less frequent following cPMd compared to cPMv conditioning. * Significant effects.

$p<0.001$). Thus, in addition to being more numerous, inhibitory effects generated by iPMd were also significantly more powerful than those originating from iPMv.

Comparison of the pattern of modulatory effects of cPMd and cPMv with each ISI

We then performed the same analyses to compare the effects of premotor areas located in the hemisphere opposite to M1. Figure 3.5C (left panel) shows the number of significant modulatory effects evoked by cPMd and cPMv conditioning in all muscles combined with each tested ISI. The graph emphasizes that the incidence of facilitatory effects was much greater following cPMd than cPMv conditioning, and this was true with each tested ISI. The difference between cPMd and cPMv was particularly striking when the C_{stim} preceded the T_{stim} by 15ms (ISI15) or when the C_{stim} and T_{stim} were delivered simultaneously (ISI0). Moreover, cPMd conditioning induced fewer inhibitory effects than cPMv with each tested ISI. The difference between the two cortical areas was the greatest when longer ISIs separated the C_{stim} and T_{stim} (ISI15 and ISI20) and with ISI0. When comparing the magnitude of the effects from cPMd and cPMv (Figure 3.5C, right panel), we found that facilitatory effects from cPMd were much more powerful than those from cPMv with all tested ISIs (Figure 3.5C, right panel), with the exception of ISI5. Facilitation from cPMd was particularly stronger with ISI15 and ISI0. While inhibitory effects were less common in cPMd compared to cPMv with all tested ISIs (Figure 53.C, left panel), the strength of inhibition was much more similar for the two premotor areas. In fact, inhibitory effects of cPMd were slightly stronger than those of cPMv with half of the tested ISIs (ISI0, ISI5 and ISI10).

Once again we combined all tested ISIs to compare the incidence of modulatory effects (Figure 3.5D, left panel) with a chi-square test (X^2), followed by a post-hoc two-proportion Z-test. We found that the distribution of modulatory effects produced by cPMd conditioning was different from that produced by cPMv ($X^2=66.6$; $p<0.001$). Conditioning stimulations in cPMd induced significantly more facilitatory effects (39.0%) (11.0%) ($p<0.001$) and significantly fewer inhibitory effects (24.8%) than in cPMv (53.0%) ($p<0.001$). To compare the magnitude of modulatory effects of cPMd and cPMv (Figure 3.5D, right panel), two-sample t-tests were used. One two-sample t-test comparing the magnitude of facilitatory effects showed that cPMd conditioning induced significantly greater facilitatory effects than cPMv (mean Z-scores=3.74 and 2.46 respectively; $t=2.70$; $p=0.008$). Another two-sample t-test comparing the magnitude of inhibitory effects showed no significant difference between cPMd and cPMv conditioning (mean Z-scores=-3.18 and -3.23

respectively; t=0.19; p=0.85). Thus, in addition to being more numerous, facilitatory effects generated by cPMd were also significantly more powerful than those originating from cPMv. In contrast, although cPMd conditioning generated fewer inhibitory effects, the magnitude of these inhibitory effects was similar to those induced by cPMv.

Comparison of the modulatory effects across ISIs from PMd and PMv

For any given MEP evoked with the T-only trials, we looked at the pattern of modulation across ISIs and separated them into 3 groups (Deffeyes et al., 2015; Quessy et al., 2016). First, the conditioning of PMd or PMv could significantly facilitate the MEP with at least one ISI, but never significantly inhibit M1 outputs with any of the ISIs (i.e. pure facilitation across ISIs). Second, the conditioning of PMd or PMv could significantly inhibit the MEP with at least one ISI, but never significantly facilitate M1 outputs with any of the ISIs (i.e. pure inhibition across ISIs). Third, the conditioning of PMd or PMv could significantly facilitate the MEP with at least one ISI and also significantly inhibit the MEP with at least one ISI (i.e. opposite effects across ISIs).

When looking at the effects across the ISIs we tested in iPMd, we did not find any MEP that was not significantly modulated with any of the ISIs, supporting that modulatory effects from iPMd on M1 outputs were very likely to occur with the ISIs we selected. Out of our population of 37 MEPs conditioned by iPMd, we found that pure facilitation (9 cases, 24.3%) was less common than pure inhibition (12 cases, 32.4%) and that opposite effects (16 cases, 43.2%) were more common than either pure facilitation or pure inhibition (Figure 3.6A). Although iPMv induced more pure facilitatory effects than either pure inhibitory or opposite effects across ISIs (Quessy et al., 2016), this pattern was not significantly different from the one induced by iPMd (X^2=2.05; p=0.72).

For cPMd conditioning, we found only 1 MEP that was not significantly modulated with any of the ISIs. This once again supports that cPMd was very likely to modulate M1 outputs with the ISIs we selected (97.6%). Out of our population of 41 MEPs, we found many more cases in which the conditioning of cPMd had pure facilitatory effects (24 cases, 58.5%), than pure inhibitory (10 cases, 24.4%) or opposite effects (6 cases, 14.6%) across ISIs (Figure 3.6B). Thus, most neural

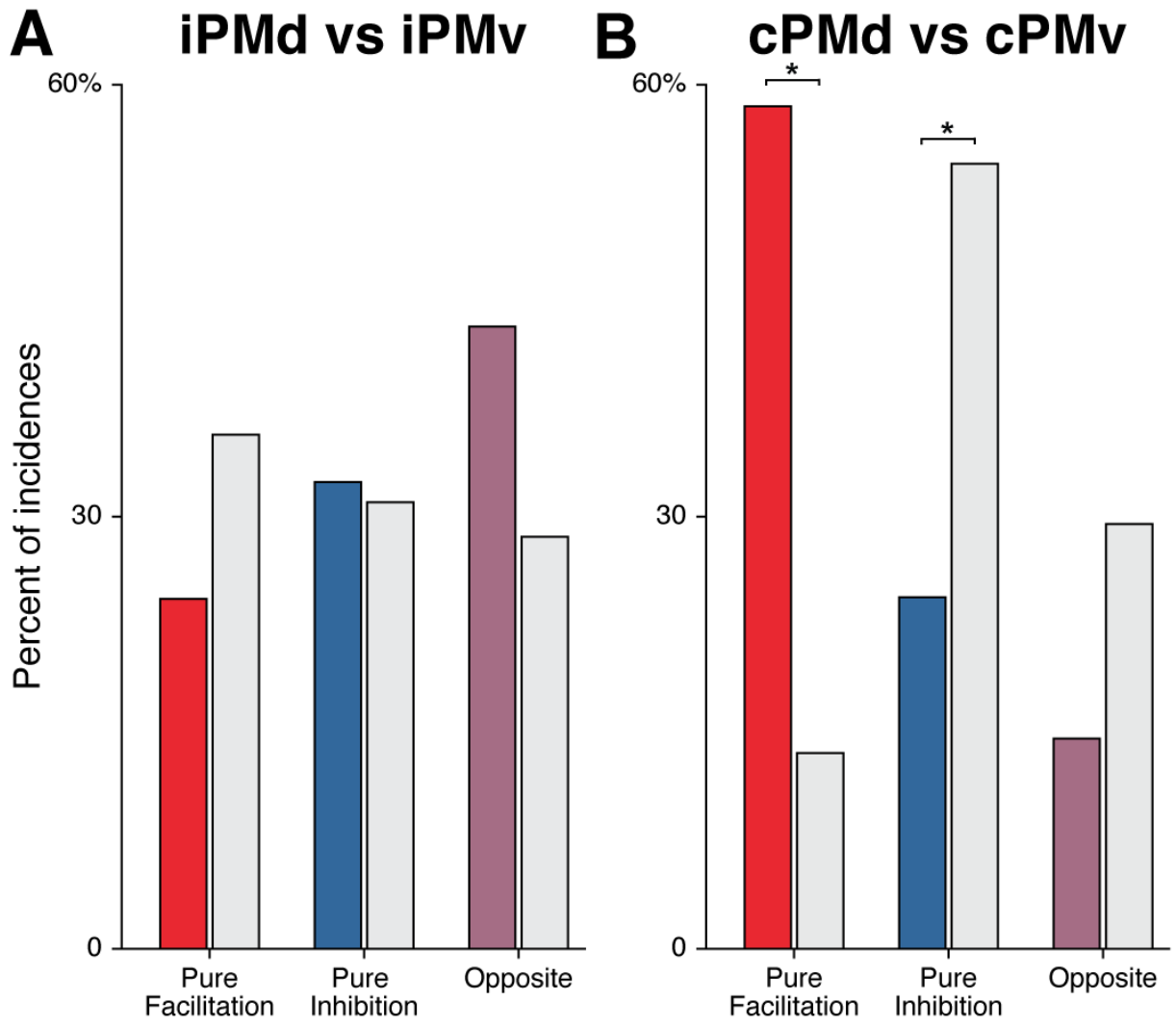

Figure 3.6 *Groups of modulatory effects across ISIs for PMd and PMv*

A) Incidence of pure facilitatory, pure inhibitory and opposite effects across ISIs for iPMd (colored bars) and iPMv (gray bars). For iPMd, there were fewer cases of pure facilitation (red) than pure inhibition (blue) and more cases of opposite effects across ISIs (purple). For iPMv, cases of pure facilitation were most common and opposite effects were the least common. However, the patterns of iPMd and iPMv across ISIs were not significantly different. This pattern was not significantly different from that induced by iPMv. **B)** Incidence of pure facilitatory, pure inhibitory and opposite effects across ISIs for cPMd (colored bars) and cPMv (gray bars). For cPMd, we found many more cases of pure facilitation (red) than pure inhibition (blue) or opposite effects (purple) across ISIs. The proportions of pure facilitation and pure inhibition induced by cPMd and cPMv were significantly different. * Significant effects.

populations stimulated in cPMd had only facilitatory effects on M1 outputs, regardless of the latency that separated the C_{stim} and T_{stim}. This pattern is quite different from the one found for cPMv (Quessy et al., 2016), which induced many more pure inhibitory effects than either opposite or pure facilitatory effects (Figure 3.6B). The pattern of effects was significantly different following cPMd and cPMv conditioning (X^2=19.1; p<0.001). Post-hoc two-proportion Z-test confirmed that cases of pure facilitation were more common following cPMd conditioning (p<0.001) and cases of pure inhibition were more common following cPMv conditioning (p=0.005). However, cPMd and cPMv conditioning induced a similar number of cases of opposite effects across tested ISIs (p=0.10). These results suggest that, in comparison to cPMv, more cortical territory in cPMd is devoted to induce pure facilitatory effects and less to induce pure inhibitory effects.

Comparison of the modulatory effects of PMd and PMv on different muscle categories

We then wanted to know if the effects of PMd and PMv conditioning were similar or different for the various muscles we recorded. To do so, we first separated the muscles into functional categories (intrinsic hand (FPB; APB), forearm extensors (ECU; EDC) and forearm flexors (PL; FDS)). We counted the incidence of significant facilitatory and inhibitory effects induced by PMd and PMv in each muscle category and compared them with a chi-square test (X^2) followed by a post-hoc two-proportion Z-test (Figure 3.7).

When looking at the effects of iPMd conditioning in different categories of muscles (Figure 3.7A), we found that facilitatory effects were most common in forearm flexors while inhibitory effects were most common in intrinsic hand muscles. The distribution of modulatory effects produced by iPMd was different from that produced by iPMv in each muscle category (intrinsic hand X^2=43.0, p<0.001; forearm extensors X^2 =17.4, p<0.001; forearm flexors X^2=10.5; p=0.01). For intrinsic hand muscles, conditioning of iPMd induced fewer facilitatory effects (29.6%) than iPMv (54.4%) (p<0.001) and more inhibitory effects (50.9% versus 10.5% for iPMd and iPMv respectively) (p<0.001). For forearm extensor muscles, conditioning of iPMd induced significantly more facilitatory effects (34.6%) than iPMv (16.7%) (p=0.005). For forearm flexor muscles, iPMd induced significantly more facilitatory effects (52.8%) than iPMv (16.7%) (p=0.001). Thus, whereas iPMd generally induced more inhibitory effects compared to iPMv (see figure 3.5B), this difference was only significant in intrinsic hand muscles.

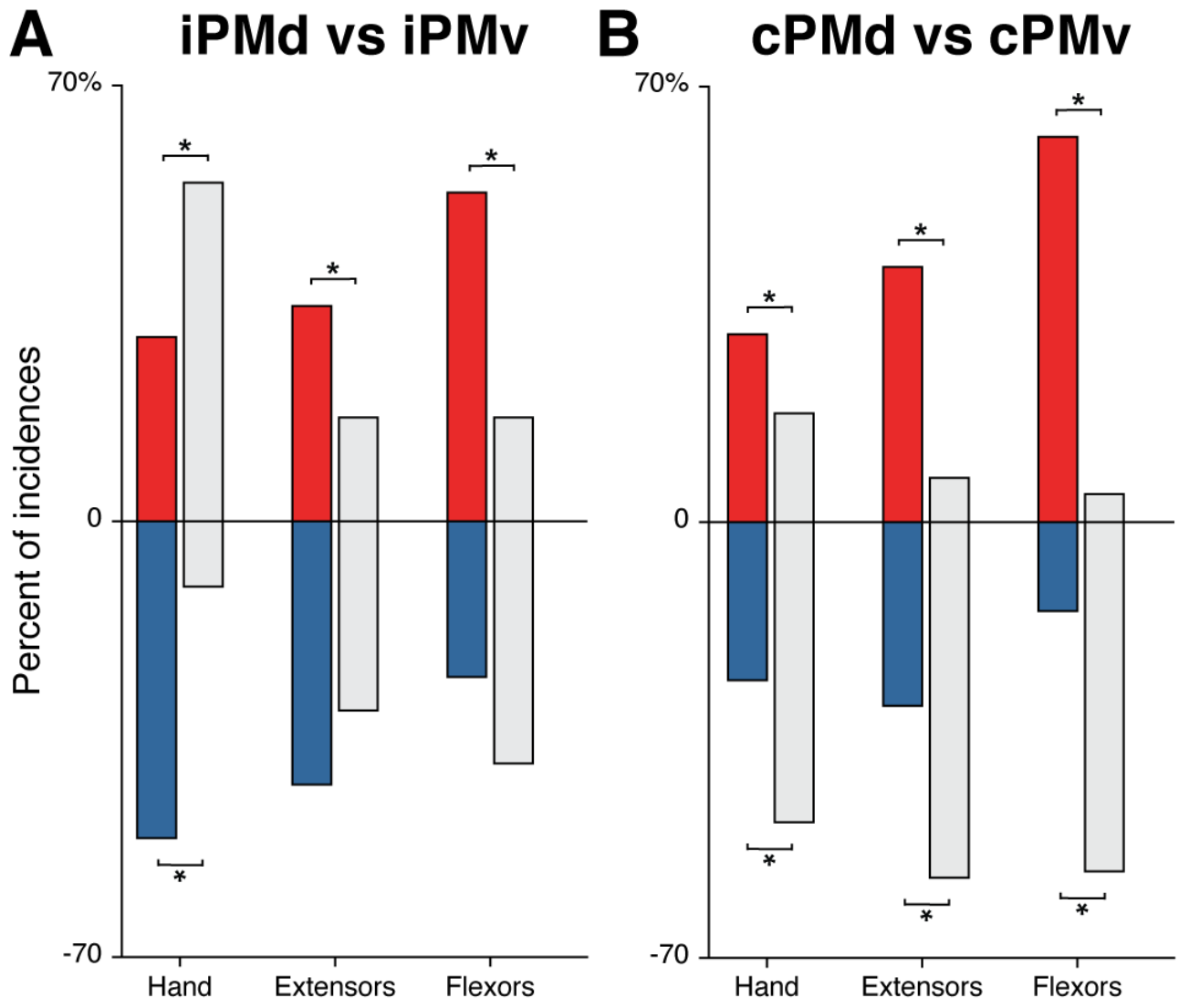

Figure 3.7. *Modulatory effects of PMd and PMv on different muscle categories*

Incidence of significant modulation induced by iPMd (colored bars) and iPMv (gray bars) in each functional category of muscles (Hand: intrinsic hand; Extensors: forearm extensors; Flexors: forearm flexors). Conditioning stimulation in iPMd induced significantly fewer facilitatory effects (red) in intrinsic hand and more facilitatory effects in forearm muscles compared to iPMv. In contrast, significant differences in incidence of inhibitory effects were only observed for intrinsic hand muscles and they were more common following iPMd conditioning (blue) compared to iPMv. **B)** Incidence of significant modulation induced by cPMd (colored bars) and cPMv (gray bars) in each functional category of muscles. Facilitatory effects were significantly more common in all 3 muscle categories following cPMd conditioning (red) in comparison to cPMv (gray). In contrast, inhibitory effects were significantly less common in all 3 muscle categories following cPMd (blue)

conditioning in comparison to cPMv conditioning. These differences between the modulatory patterns of cPMd and cPMv were more pronounced in forearm muscles, especially in flexor muscles. * Significant effects.

For the effects of cPMd in different categories of muscles (Figure 3.7B), we found that facilitatory effects were most common in forearm muscles, especially in flexors, while inhibitory effects were most common in forearm extensors. Once again, this pattern was different from that produced by cPMv for all 3 muscle categories (intrinsic hand X^2=14.1; p=0.002; forearm extensors X^2 =27.3; p<0.001; forearm flexors X^2=44.6; p<0.001). In intrinsic hand muscles, conditioning of cPMd induced significantly more facilitatory effects (30.2%) than cPMv (17.5%) (p=0.02) and fewer inhibitory effects (25.4% versus 48.2% for iPMd and iPMv respectively) (p<0.001). Similar, but even more pronounced differences were observed in forearm muscles. For forearm extensor muscles, conditioning of cPMd induced significantly more facilitatory effects (41.0%) than cPMv (7.1%) (p<0.001) and fewer inhibitory effects (29.5% versus 57.1% for iPMd and iPMv, respectively) (p<0.001). For forearm flexor muscles, cPMd induced significantly more facilitatory effects (61.9%) than cPMv (4.5%) (p<0.001) and fewer inhibitory effects (14.3% and 56.1% for iPMd and iPMv, respectively) (p<0.001). Thus, the differences of modulatory effects from cPMv and cPMd were less pronounced in intrinsic hand muscles and more pronounced in forearm muscles, especially in flexors.

Comparison of the modulatory effects across muscles from PMd and PMv

We then wondered if the conditioning stimulation had the same or different effects across muscles we recorded. In theory, the conditioning stimulation could induce significant facilitation on the MEP of one and up to all 6 muscles (i.e. pure facilitation across muscles), could only be inhibitory on the MEPs (i.e. pure inhibition across muscles), or simultaneously facilitate and inhibit different combinations of muscles (i.e. simultaneous mixed effects across muscles) (Deffeyes et al., 2015; Quessy et al., 2016). For each protocol (i.e. cortical stimulation site), we counted the incidence of each of these 3 possible groups of effects across muscles (Figure 3.8).

For protocols recorded with iPMd conditioning (12 protocols x 6 ISIs=72 cases), we found 25 cases (35.7%) in which iPMd evoked only facilitation across recorded muscles and in 19 of these (76.0%), more than one muscle was simultaneously facilitated. We found considerably more cases in which iPMd evoked only inhibition across recorded muscles (40 cases; 55.6%) and in 30 of these (75.0%), more than one muscle was simultaneously inhibited. Finally, we rarely found cases in which iPMd induced simultaneous mixed effects across muscles (2 cases; 2.8%) (Figure

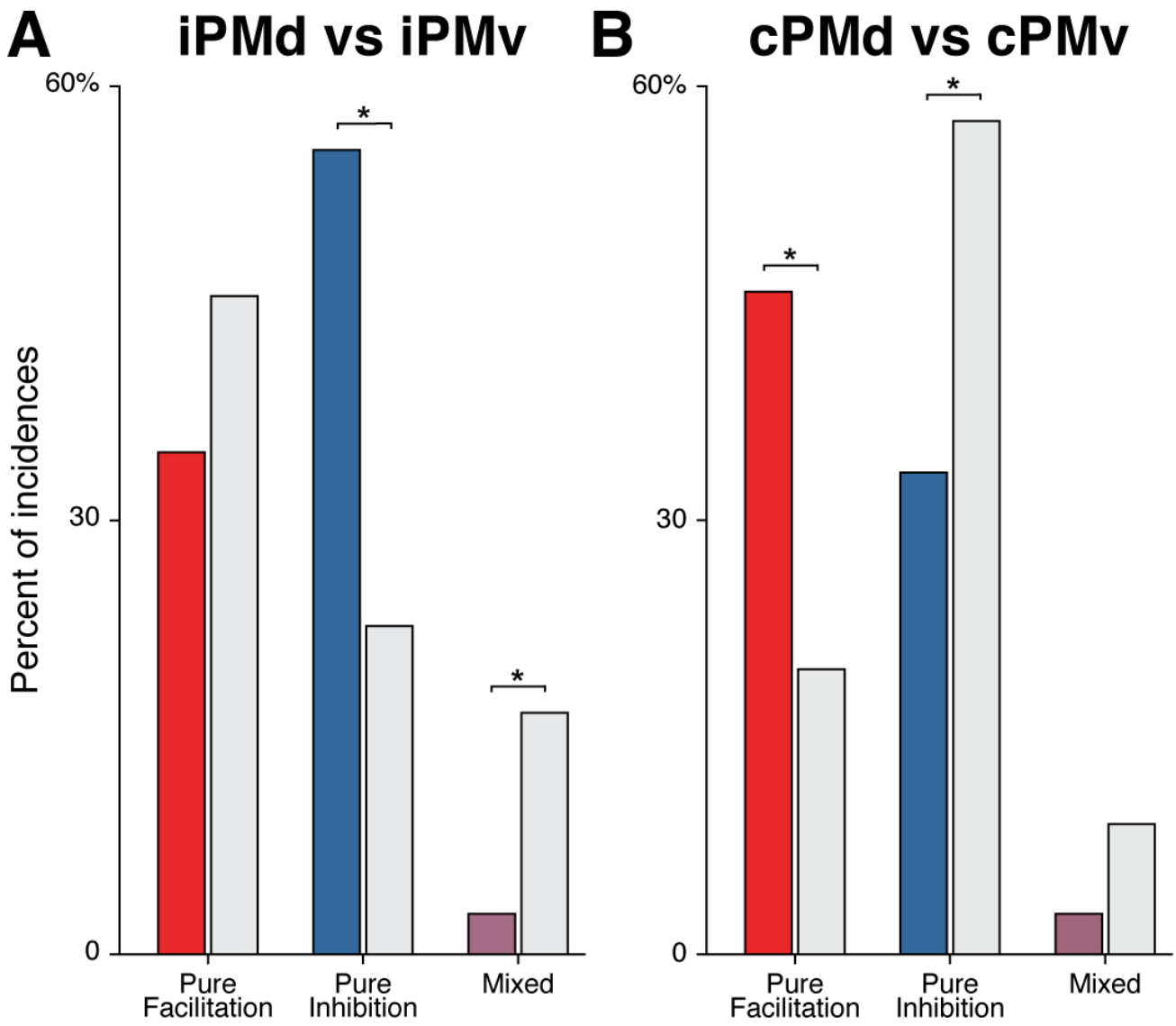

Figure 3.8 *Groups of modulatory effects across muscles for PMd and PMv*

A) Incidence of pure facilitatory, pure inhibitory and mixed effects across muscles for iPMd (colored bars) and iPMv (gray bars). Each bar represents the proportion of cases in which a given paired-pulse protocol (i.e. interactions between two cortical sites) induced pure facilitatory, pure inhibitory or mixed effects across the various muscles modulated with a given ISI. We found that iPMd induced significantly more cases of pure inhibitory effects (blue) and fewer cases of mixed effects (purple) across muscles than iPMv. **B)** Incidence of pure facilitatory, pure inhibitory and mixed effects across muscles for cPMd (colored bars) and cPMv (gray bars). We found that cPMd induced significantly more cases of pure facilitatory effects (red) and fewer cases of pure inhibitory effects (blue) across muscles than iPMv. * Significant effects.

3.8A). This pattern was different than what we previously found for iPMv (X^2=17.4; p<0.001) (Quessy et al., 2016). Post-hoc two-proportion Z-tests revealed that iPMd and iPMv conditioning induced similar amounts of pure facilitatory effects (p=0.25). However, iPMd induced more cases of pure inhibition than iPMv (p<0.001) while iPMv induced more cases of simultaneous mixed effects across muscles than iPMd (p=0.01).

Finally, for protocols recorded with cPMd conditioning (Figure 3.8B; 12 protocols x 6 ISIs=72 cases), we found 33 cases (45.8%) of pure facilitation across muscles and in 23 of these (69.7%), more than one muscle was facilitated. We found fewer cases of pure inhibition (24 cases; 33.3%) and in 20 of these (83.3%), more than one muscle was inhibited. We rarely found cases of simultaneous mixed effects (2 cases; 2.8%). Once again, this pattern was quite different than what we previously found for cPMv (Quessy et al., 2016) (X^2=13.9; p=0.002). Post-hoc two-proportion Z-tests confirmed that in comparison to cPMv, the incidence of cases of pure facilitation was greater (p=0.001) and the incidence of pure inhibition across muscles was smaller (p=0.004) after cPMd conditioning. However, cPMd and cPMv conditioning induced comparable amounts of simultaneous mixed effects across muscles (p=0.11).

Discussion

Our objectives were to study the modulatory effects of PMd on the outputs of M1 to intrinsic hand and forearm muscles with invasive miscrostimulations techniques and compare them to those of PMv collected in the same animals (Quessy et al., 2016). We found that iPMd was more likely to inhibit M1 outputs than iPMv, and these inhibitory effects were more powerful. In the opposite hemisphere, cPMd was more likely to facilitate M1 outputs than cPMv and these facilitatory effects were more powerful. Our results support that the patterns of modulations induced by PMd and PMv are strikingly different. These contrasting effects could support the specific roles these premotor areas play for the production of hand movements and may predispose them to contribute differently to the reorganization of cortical networks after brain injury.

Invasive microstimulations to study interactions of cortical outputs

Most studies investigating the interactions of cortical outputs are conducted with TMS. While there are several advantages to TMS, such as the ease to test interactions in various behavioral contexts, one limitation is that the volume of stimulated tissue is relatively large (~1cm) (Cowey, 2005; Wassermann et al., 2008). Consequently, only effects of broad populations of neurons from a few distinct cortical sites can be effectively investigated from each area. Furthermore, the size of the coils may be a problem for paired-pulse paradigms when the two tested areas are in close proximity, such as for iPMd and M1. In the present study, the use of ICMS with intensities ≤300μA allowed us to stimulate much smaller cortical volumes (<0.5mm radius) (Stoney et al., 1968) to reveal how clusters of neurons within iPMd and cPMd can affect M1 outputs. Across all protocols, the closest pair of C and T electrodes were <5.6 mm apart (sites C10 and T10 in CB2), insuring that the effects induced by iPMd were not due to current spread to M1.

We chose to collect our data in terminal preparations under sedation (Cerri et al., 2003; Quessy et al., 2016). An advantage of these preparations is that a great quantity of data can be collected within a single experiment under stable conditions. Here, it allowed the sampling of several cortical sites and testing of multiple ISIs. These experiments can be viewed more as 'neuroanatomical', providing insights into the range of potential effects the outputs of iPMd and cPMd can exert on M1 through the different pathways these cortical areas share. However, the

reciprocal nature of connections between premotor areas and M1 should also be kept in mind (Dum and Strick, 2005; Dancause et al., 2006b; Dea et al., 2016; Hamadjida et al., 2016). We cannot exclude that some of the observed effects were caused by antidromic activation of M1 neurons projecting to PMd.

The diversity or variability of modulations we found across tested sites is in line with previous reports using ICMS techniques in monkeys (Tokuno and Nambu, 2000; Prabhu et al., 2009) and appears to be an inherent property of premotor areas' effects on M1 neurons and M1 outputs. We propose that it provides a versatile substrate for premotor areas to contribute to a wide variety of motor functions. During different stages of movement preparation and production or depending on the task, the variability of modulatory effects may decrease as different sub-populations of premotor neurons are selectively activated and exert the prominent influence on motor outputs. This state-dependent selective activation of different circuits in awake behaving animals could explain differences with the patterns of modulatory effects observed under sedation (e.g. see (Cerri et al., 2003; Prabhu et al., 2009)).

Modulatory effects of iPMd and cPMd on the outputs of M1

TMS studies in humans have shown that iPMd can induce either facilitatory or inhibitory effects on M1 outputs to intrinsic hand muscles, depending on the timing between the stimulations (ISIs) and the conditioning stimulation intensity (Civardi et al., 2001; Koch et al., 2007; Groppa et al., 2012). Using ICMS techniques in cebus monkeys, we also found that the proportion of facilitation and inhibition induced by iPMd was greatly affected by ISIs. While facilitation was much more common with shorter ISIs, inhibition was much more likely to be induced with longer ISIs. Noticeably, facilitatory effects were particularly common and powerful when the C_{stim} and T_{stim} were applied simultaneously (ISI0).

Based on the estimated intrahemispheric conduction time between premotor areas and M1 (~1-2ms) (Godschalk et al., 1984; Tokuno and Nambu, 2000), it is tempting to speculate that facilitatory effects from iPMd with ISI0 could, at least in part, be carried through sub-cortical routes. In particular, corticospinal projections of iPMd to lower cervical levels (He et al., 1993), where motoneurons controlling distal forearm muscles are located, could favor the integration of

facilitatory outputs from iPMd and M1 to intrinsic hand and forearm muscles in the spinal cord. Interestingly, PMv has many fewer direct projections to lower cervical segments (He et al., 1993; Borra et al., 2010), which may favor the cortico-cortical route.

Studies in humans using TMS have also reported that cPMd can have both facilitatory (Baumer et al., 2006) and inhibitory (Mochizuki et al., 2004) effects on M1 outputs to intrinsic hand muscles at rest, depending on the C_{stim} intensity (Koch et al., 2006). Shifts between facilitation and inhibition also occur during different phases of movement preparation and production (Kroeger et al., 2010; Liuzzi et al., 2010). Our results show, similarly, that cPMd can be both facilitatory and inhibitory on M1 outputs.

While the modulatory effects of cPMd induced with most of the ISIs we tested could have been carried through callosal projections, we also found several cases in which cPMd modulated M1 outputs with ISI0. Considering an interhemispheric conduction time of ~2-6ms (Asanuma and Okuda, 1962; Matsunami and Hamada, 1984), it is unlikely that these effects were caused by cortical interactions. They may rather have occurred at a downstream site of convergence, for example through bilateral projections of premotor areas and M1 to the reticular formation (Kuypers and Lawrence, 1967; Keizer and Kuypers, 1989; Kably and Drew, 1998).

Comparison of the pattern of modulatory effects from PMd and PMv on M1 outputs

The main finding of the present study is the sharp contrast between modulatory effects from PMd and PMv. In the ipsilateral hemisphere, iPMd induced more and stronger inhibitory effects on M1 outputs than iPMv. Studies in both humans and monkeys have demonstrated that iPMd is involved in intersegmental coupling and monitoring of the different components of prehensile movements (Raos et al., 2004; Davare et al., 2006). In this context, it is possible that inhibition induced by iPMd suppresses unwanted outputs, decreases co-contractions and refines the coordination of EMG patterns across the entire arm. In contrast, iPMv seems primarily involved in transforming object properties into proper hand configurations for grasping (Fogassi et al., 2001; Davare et al., 2006). Powerful facilitation from iPMv may thus be used to favor the outputs to the appropriate muscles to form adapted grips (Prabhu et al., 2009).

In the contralateral hemisphere, cPMd had more and stronger facilitatory effects on M1 outputs than cPMv. Human imaging studies have shown that the activity in cPMd increases with the complexity of sequential finger movements (Sadato et al., 1996) and that cPMd is involved in the execution of complex bimanual anti-phase movements (Meyer-Lindenberg et al., 2002). Hence, facilitatory effects from cPMd may be used to strengthen the most efficient M1 activation patterns to produce dexterous movements of the ipsilateral hand (Horenstein et al., 2009) or help to maintain synchronous asymmetrical movements of the two hands (Liuzzi et al., 2011). In comparison, inhibitory effects from cPMv may help to suppress mirror movements in the arm contralateral to M1 to favor unimanual grasping (Nudo and Masterton, 1990; Wise, 2006).

Finally, the contrasting pattern of modulatory effects from cPMd and cPMv may also have major implications for motor recovery after brain injury. Most neuromodulatory protocols currently tested in stroke patients attempt to inhibit the contralesional M1 based on the concept of interhemispheric imbalance (Nowak et al., 2009). According to this view, the predominant inhibitory effects from contralesional M1 on the ipsilesional network increase after stroke (Murase et al., 2004; Duque et al., 2005a) and interfere with recovery. However, the current model of interhemispheric imbalance does not consider the impact of other cortical areas in the complex motor network of primates and is likely oversimplified (Grefkes and Fink, 2012).

We propose that the contrasting patterns of modulatory effects of cPMd and cPMv could predispose them to play different, somewhat opposing roles in post-stroke interhemispheric interplay and recovery. In addition to M1, cPMv may also exert detrimental inhibition on the ipsilesional network in some patients and cPMd could play a favorable role towards the reestablishment of interhemispheric balance. If so, inhibition of cPMv or facilitation of cPMd could be more effective strategies in these patients. While the potential of these approaches will have to be investigated in future experiments, our data certainly point out that cPMd and cPMv should be considered as prime targets for the development of alternative neuromodulatory treatments.

Chapitre 4 – Modulatory effects of the supplementary motor area (SMA) on primary motor cortex outputs

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Abstract

Premotor areas of primates are specialized cortical regions that can contribute to hand movements by modulating the outputs of the primary motor cortex (M1). The goal of the present work was to study how the supplementary motor area (SMA) located within the same hemisphere (i.e. ipsilateral SMA or iSMA) or the opposite hemisphere (i.e. contralateral or cSMA) modulate the outputs of M1. We used paired-pulse protocols with intracortical stimulations in sedated cebus monkeys. A conditioning stimulus in iSMA or cSMA was delivered simultaneously or prior to a test stimulus in M1 with different interstimulus intervals (ISIs) while recording electromyographic activity in hand and forearm muscles. The pattern of modulation from iSMA and cSMA shared some clear similarities. In particular, both areas predominantly induced facilitatory effects on M1 outputs with shorter ISIs and inhibitory effects with longer ISIs. However, the incidence and strength of facilitatory effects was greater for iSMA than cSMA. We then compared the pattern of modulatory effects from SMA to the ones from the dorsal and ventral premotor cortices (PMd and PMv) collected in the same series of experiments. Among premotor areas, the impact of SMA on M1 outputs was always weaker than the one of either PMd or PMv, and this regardless of the hemisphere, or the ISI tested. These results show that SMA exerts a unique set of modulations on M1 outputs, which could support its specific function for the production of hand movements.

Introduction

The refinement of manual skills and the development of complex motor behaviors in primates are associated with the appearance of several premotor areas including the supplementary motor area (SMA), the dorsal premotor and the ventral premotor cortex (PMd and PMv, respectively) (Kaas, 2006). These premotor areas are major nodes of functionally specialized sensorimotor networks involved in different aspects of hand motor control. For example, while SMA seems particularly concerned with movement sequences, PMd appears mainly involved in monitoring and coupling the different phases of prehensile movements and PMv in preshaping the hand to match the features of objects to be grasped (Shima and Tanji, 1998, 2000; Fogassi et al., 2001; Raos et al., 2004; Davare et al., 2006). The complex functions associated with each premotor area support the idea that these specialized motor regions have emerged to sustain the increased behavioral repertoire of the primate's hand (Hamadjida et al., 2016). However, it is still unclear how the information processed in premotor areas gives rise to the muscle activation patterns required to execute this vast repertoire.

One way premotor areas can uniquely contribute to the production of hand movements is by modulating or shaping the outputs of the primary motor cortex (M1) differently. Several studies in both humans and monkeys have examined this 'physiological connectivity' between premotor areas and M1 using dual site, paired-pulse protocols with transcranial magnetic stimulation (TMS) and intracortical microstimulation (ICMS). Such experiments reveal how premotor areas can enhance (facilitate) or suppress (inhibit) descending motor outputs from M1 and thus provide information about how these areas can contribute to the production of movements. Most of these studies have focused on the modulatory effects of either PMd (Civardi et al., 2001; Mochizuki et al., 2004; Koch et al., 2006) or PMv (Cerri et al., 2003; Davare et al., 2008; Prabhu et al., 2009). Using paired-pulse ICMS in cebus monkeys, we recently compared the modulatory effects of PMd and PMv on the outputs of M1 directly, in the same animals and experimental conditions, which revealed highly contrasting patterns of modulation (Quessy et al., 2016; Côté et al., 2017). In the ipsilateral hemisphere, PMd induces more frequent and more powerful inhibitory effects than PMv. The opposite trend was found in the contralateral hemisphere, in which PMd induces more frequent and more powerful facilitatory effects than PMv.

While the physiological connectivity of PMd and PMv with M1 has been investigated in some detail, relatively few studies have examined the modulatory effects of SMA (Oliveri et al., 2003; Arai et al., 2012; Fiori et al., 2017). Those paired-pulse TMS experiments conducted in humans have shown that SMA can both facilitate and inhibit the outputs of M1 to hand muscles, depending on the timing used between the stimulations. However, because SMA is buried along the medial wall, it is technically challenging to unequivocally isolate stimulations to either the ipsilateral SMA (iSMA) or contralateral SMA (cSMA) with TMS (Fiori et al., 2017). Consequently, the modulatory profile specific to iSMA and cSMA remains poorly understood. Moreover, because various experimental designs have been used across human TMS studies, we currently have an incomplete understanding of how modulatory effects induced by SMA compare to those of PMd or PMv.

To address some of these issues, we employed paired-pulse ICMS protocols to examine the modulatory effects of iSMA and cSMA on M1 outputs in cebus monkeys. An important advantage of ICMS is that stimulations are confined to one hemisphere, which allows us to distinguish the modulatory influences of iSMA and cSMA. We then compared the modulatory effects of SMA to those of PMd and PMv collected in the same series of experiments (Quessy et al., 2016; Côté et al., 2017). Given the different roles assumed by each premotor area with regards to hand movements, we hypothesized that they would induce distinct patterns of modulation on M1 outputs.

Methods

Ethical approval

Our experimental protocol was in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Comité de Déontologie de l'Expérimentation sur les Animaux (CDEA) of the Université de Montréal.

Subjects

Two adult female capuchin monkeys (*Sapajus apella*; CB3 (1.4kg) and CB4 (1.2kg)) purchased from Alpha Genesis Inc. (Yemassee, SC, USA) were used in this study. These animals were also part of our experiments in which we studied the modulatory effects of PMd and PMv (Quessy et al., 2016; Côté et al., 2017) in four adult female capuchin monkeys (CB1, CB2, CB3 and CB4). For CB3 and CB4, data for SMA was collected in the same procedure as the ones for PMd and PMv. Animals were housed with ad libitum food and water.

Surgical Procedures

All procedures were performed in a terminal experiment. Anaesthesia was induced with an intramuscular injection of ketamine (15 mg/kg; Ketaset; Pfizer, Inc, New York, NY, USA) and transitioned to isoflurane (~2% in 100% O_2; Furane; Baxter, Deerfield, IL, USA). The animal was placed in a stereotaxic frame and received an intramuscular injection of Dexamethasone 2 (Vetoquinol®; 0.5 mg/kg) and an intravenous injection of Mannitol 20% (1500 mg/kg). A continuous infusion of lactated ringer's solution was delivered intravenously (10 ml/kg/h) and vital signs (heart rate, respiration rate, arterial oxygen saturation and body temperature) were monitored throughout the surgery. At the end of the experiment, the animal was euthanized with a lethal dose of pentobarbital (Euthansol; 100mg/kg).

In both monkeys, 8 muscles in each arm were implanted intramuscularly with insulated multistranded microwires to record EMG activity (Cooner Wire; Chatsworth, CA, USA) (flexor pollicis brevis (FPB), abductor pollicis brevis (APB), extensor carpi ulnaris (ECU), extensor digitorum communis (EDC), palmaris longus (PL), flexor digitorum superficialis (FDS), biceps

brachii (BB) and triceps brachii (TB)). A craniotomy and durectomy were performed on the right hemisphere to expose M1 and iSMA and on the left hemisphere to expose cSMA.

Paired-pulse stimulation and EMG recording

For the acquisition of electrophysiological data, isoflurane was withdrawn and sedation was maintained with intravenous injections of Ketamine (Ketaset; ~10 mg/kg/10 minutes) and Diazepam (Valium; ~0.01mg/kg/hr). To identify suitable cortical sites for paired-pulse protocols, we first located the hand representations in M1, iSMA and cSMA using standard ICMS trains (13 monophasic cathodal pulses of 0.2ms delivered at 350Hz) delivered at 1Hz (Dancause et al., 2008; Dea et al., 2016; Hamadjida et al., 2016) (Figure 4.1A). Only cortical sites inducing clear EMG responses in at least one contralateral intrinsic hand or forearm muscle with ICMS trains were retained for paired-pulse protocols. The cortical sites chosen for SMA were all located caudal to the genu of the arcuate sulcus, in the region of SMA highly interconnected with M1 in cebus monkeys and where corticospinal neurons are located (He et al., 1995; Dum and Strick, 2005; Akkal et al., 2007; Dea et al., 2016; Hamadjida et al., 2016). Hence, all electrophysiological data were collected from distal forelimb representations, as characterized by short ICMS trains, in the caudal subdivision of SMA (i.e. SMA proper).

For paired-pulse protocols, we used two single wire insulated tungsten electrodes (FHC; Bowdoin, ME, USA) positioned with two independent micromanipulators (David Kopf instruments; Tujunga, CA, USA). The electrode delivering the conditioning stimulation (C_{stim}) was placed in the distal forelimb representation of either iSMA or cSMA (depth ~3000-4000µm) while the electrode delivering the test stimulation (T_{stim}) was positioned in the distal forelimb representation of M1 (depth ~1800µm) (Figure 4.1A). Both stimulations consisted of a single cathodal square pulse of 0.2ms duration. To establish the current intensities for the C_{stim} and T_{stim}, the threshold current intensity (current at which EMG activity was evoked by ~50% of single pulses; ≤ 300µA) was determined online using the EMG activity recorded in muscles of the arm contralateral to each electrode. Single-pulse stimulation in iSMA and cSMA did not evoke clear responses with current intensity of up to 300µA. We thus arbitrarily set the C_{stim} intensity to 225µA for all cortical sites included in the present study. A similar intensity was used for the C_{stim} in our previous studies for cortical sites that did not evoke motor evoked potentials (MEPs) with single

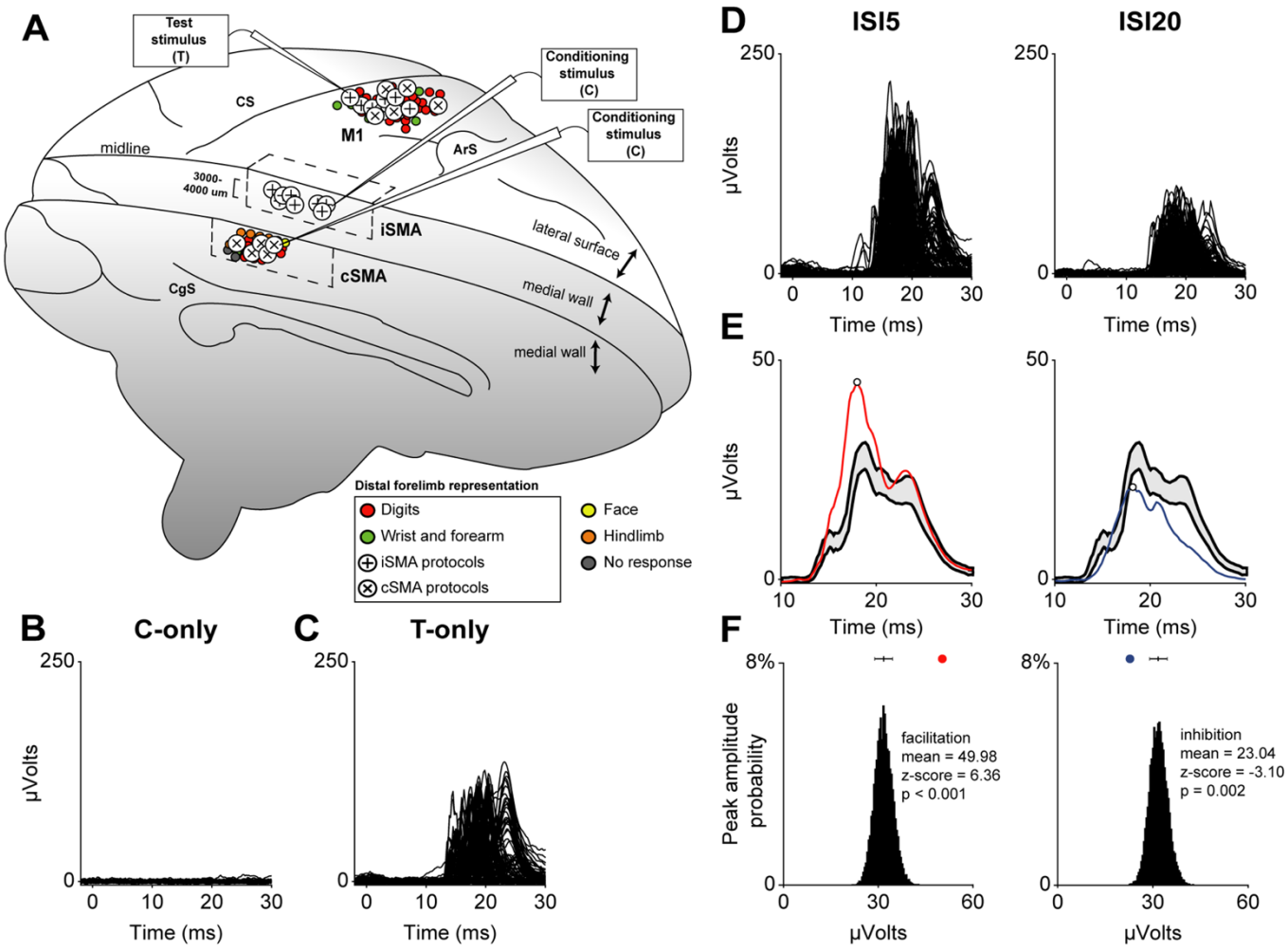


Figure 4.1 *Experimental methods*

A) Schematic representation of the experimental setup. Motor maps derived with ICMS trains (small dots color-coded according to the legend below) and locations of cortical sites used for paired-pulse protocols (large circles) are shown. The cortical sites used for the T_{stim} were located in the hand representation of M1 and the cortical sites for the C_{stim} in the ipsilateral SMA or contralateral SMA (iSMA and cSMA, respectively). Large circles with + symbols show cortical locations of the electrodes used for ipsilateral protocols and large circles with x symbols show cortical locations of the electrodes used for contralateral protocols. All cortical sites selected were located rostral to the hindlimb representation, at depths ~3000-4000µm along the medial wall. **B)** Example of single C-only trials recording in APB for a given protocol, in which single pulse stimulations with the conditioning electrode was placed in cSMA. Because the stimulation intensity was sub-threshold, no clear response is observed. **C)** Example of responses induced in APB with

T-only trials during the same protocol. **D)** Examples of responses induced with single C+T trials in the same muscle, still during the same protocol. Here we show responses evoked with ISI5 (left panel) and ISI20 (right panel). **E)** Mean responses obtained from the single trials in D (ISI5: left panel, red trace; ISI20 : right panel, blue trace) in relation to the ± one standard deviation of the predicted MEP (gray area and black traces). The open circles on the colored traces show the peaks of the mean responses of the paired stimulation. **F)** Probability distribution of predicted peak amplitudes (n=10,000). The histograms show the probability of occurrence (y axis) of predicted peaks with different amplitudes (x axis). The black line and whiskers above the histograms indicate the mean and standard deviation of the probability distribution. The colored dots show the values of the average peak amplitude obtained with ISI5 (red) and ISI20 (blue) from the traces in E. The average peak amplitude with ISI5 was greater than the probability distribution (Z-score=6.36; $p<0.001$) and was thus considered as a significant facilitation. The average peak amplitude with ISI20 was smaller than the probability distribution (Z-score=-3.10; $p=0.002$) and was thus considered as a significant inhibition.

pulse stimulations (Quessy et al., 2016; Côté et al., 2017). The current intensity used for the single-pulse T_{stim} in M1 was generally set to 125% of threshold (range=85-300µA, mean=199µA). However, if the evoked EMG activity was too small or too large with this value, the intensity was adjusted to produce clear, but submaximal responses. This ensured that the responses evoked by the T_{stim} could be either increased or decreased by the C_{stim}.

After positioning the electrodes and establishing appropriate stimulation intensities, a paired-pulse stimulation protocol was initiated. Within a protocol, stimulations could be delivered through the conditioning electrode only (C-only trials), the test electrode only (T-only trials), or through both electrodes (paired stimulations or paired-pulse trials; C+T) using one of 6 different inter-stimulus intervals (ISIs). When the C_{stim} was in iSMA, the paired stimulations were delivered simultaneously (ISI0) or with ISIs of 1ms (ISI1), 2ms (ISI2), 4ms (ISI4), 6ms (ISI6) or 10ms (ISI10). When the C_{stim} was in cSMA, the paired stimulations were delivered simultaneously (ISI0) or with ISIs of 2.5ms (ISI2.5), 5ms (ISI5), 10ms (ISI10), 15ms (ISI15) or 20ms (ISI20). For each of the 8 stimulation conditions (i.e. C-only, T-only and the 6 paired-pulse conditions), 150 trials delivered at 3Hz were collected (total number of trials per protocol=1200). The condition used for subsequent trials was randomly selected.

The reasoning behind the choice of these ISIs has been extensively discussed in a previous paper (Côté et al., 2017). Briefly, ISI0 may favor convergent projections of SMA and M1 at common downstream targets, for example in the spinal cord or the brainstem. For example, corticospinal volleys from SMA can reach cervical levels approximately 0.4ms after outputs from M1 (Maier et al., 2002), a short timing difference that should allow for interaction of the two outputs at the spinal cord level. Short ISIs (ISI1 and ISI2 for iSMA and ISI2.5 and ISI5 for cSMA) may favor direct projections from SMA to M1 and long ISIs (ISI4, ISI6 and ISI10 for iSMA and ISI10, ISI15 and ISI20 for cSMA) oligosynaptic projections from SMA to M1 or common targets of the two areas. It should however be kept in mind that this experimental design does not allow to pinpoint the pathways through which the modulatory effects occur with each ISI. Also, we cannot exclude the possibility that short latency effects could include modulations caused by antidromic activation of M1 neurons projecting to SMA as both areas are highly interconnected (Dum and Strick, 2005; Dea et al., 2016; Hamadjida et al., 2016). Moreover, short latency effects could be

mediated by orthodromic modulations of I-waves generated by the T stimulation in M1 (Maier et al., 2002; Cerri et al., 2003; Shimazu et al., 2004).

Once data collection was completed for a protocol, the two electrodes were moved to different cortical positions and another protocol was initiated. A total of 14 protocols were conducted with the C_{stim} in iSMA and 11 protocols with the C_{stim} in cSMA. As EMG activity was simultaneously recorded from 8 muscles, we collected 112 EMG recordings for iSMA (14 protocols x 8 muscles) and 88 EMG recordings for cSMA (11 protocols x 8 muscles) under 8 conditions (iSMA=total of 896 recordings; cSMA=total of 704 recordings). Similar to our previous studies (Quessy et al., 2016; Côté et al., 2017), we found that MEPs were stable throughout data collection by comparing the responses obtained with the T-only trials from the first 75 trials to those obtained with the last 75 trials (t=-1.36; p=0.50).

Paired-pulse stimulation protocols as well as EMG recordings were monitored via a RZ5 real-time processor (Tucker Davis Technologies (TDT); Alachua, FL, USA) with custom designed software. One part of the software regulated the stimulations generated by an IZ2 stimulator (Tucker Davis Technologies (TDT); Alachua, FL, USA) and the other part regulated EMG data acquisition. Each EMG channel was recorded at 4.9 kHz. EMG data were stored for offline analysis.

Electromyographic (EMG) data analysis

Data were analyzed offline with custom written MatLab (Version R2014a; Nantick, MA, USA) code. The continuous raw EMG recordings were separated into individual trials (n=1200) and aligned to the end of the last stimulation (i.e. the C_{stim} for C-only trials and the T_{stim} for the T-only trials and trials with the 6 paired-pulse conditions). The raw EMG signals were then analyzed in a window of 30ms after the end of the stimulation (Figure 4.1B-D), full-wave rectified and smoothed using a 5-point moving average (window=1.02ms).

We first confirmed that C_{stim} alone did not evoke EMG responses in all recorded muscles. This validates the assumption of linear summation for the calculation of our predictor (Baker and Lemon, 1995) (see below). Then, we determined whether the T_{stim} alone (T-only trials) in M1 could produce a clear MEP and that this response was large enough to detect potential increases or

decreases of activity due to paired-pulse stimulations (C+T) (Quessy et al., 2016). To do so, we compared the average baseline activity to the average MEP peak amplitude obtained from the T-only trials (n=150). To be kept for further analyses, the average MEP peak amplitude of the T_{stim} had to be greater than 3 standard deviations (SD) above the average baseline.

To analyze the modulation of significant T_{stim} MEPs by the C_{stim}, we compared the average MEP peak amplitude of paired-pulse trials (C+T) with each ISI (n=150 per ISI) to a probability distribution of predicted peak amplitudes based on the linear summation of responses in C-only and T-only trials (Figure 4.1E-F). This approach was described in detail in a previous study (Quessy et al., 2016). Briefly, the first step was to linearly sum all possible combinations (n=22,500) of single C-only traces (n=150) with single T-only traces (n=150). Among this population of predicted traces, we randomly sampled 150 trials and averaged them to produce an average predicted MEP. The peak amplitude of the average predicted MEP was calculated (peak maximum-peak minimum voltage value) within a 30ms window after the end of the stimuli. This process was repeated 10,000 times to produce a probability distribution of predicted peak amplitudes. In order to accurately identify the response peak, we removed the stimulus artifact in some T-only and C-only traces (5.4% of all traces) by replacing the activity occurring 2ms after the stimulation by the average baseline activity (30ms window before the stimulation onset). The average MEP peak amplitude of paired-pulse trials with each ISI was then compared to the probability distribution to establish the direction of the modulation (facilitation or inhibition). The normalized strength of the modulation was then obtained by calculating the Z-score of the average MEP peak amplitude of paired-pulse trials with each ISI. To be considered significant, the average MEP peak amplitude of paired-pulse trials had to differ from the mean of the distribution of predicted peak amplitudes by more than 1.96 SD ($p \leq 0.05$).

Comparison of modulatory effects of SMA to the ones from PMd and PMv

We compared the modulatory patterns of SMA reported in the present study to those of PMd and PMv collected in the same series of experiments (total number of cortical sites: SMA=25, PMd=24; PMv=22) (Quessy et al., 2016; Côté et al., 2017). To do so, we wanted to combine the incidence and magnitude of modulatory effects from each premotor area into a single measure (Zaaimi et al.,

2012) that reflects its potential impact on M1 outputs. Impact scores were calculated separately for facilitatory and inhibitory effects according to the following formula:

$$Impact\ score = \frac{\sum_{i=1}^{n}(incidence\ of\ significant\ modulation\ \times\ magnitude\ of\ modulation\ of\ MEPi)}{n}$$

in which *n* is the number of significant modulations for a studied interaction. The formula was used to calculate impact scores with each ISI and also to provide a global impact score that combined data across ISI for a given cortical area. For example, with ISI0 iSMA induced 16 significant facilitations (i.e. n=16) out of a total of 57 conditioned MEPs. The magnitude of each of the significant 16 facilitatory effects was multiplied by 0.28, the incidence of significant facilitations with ISI0, and these values were then averaged to give the faciliatory impact score of iSMA with ISI0. For the global facilitatory impact of iSMA, we found 102 significant facilitations with all ISIs tested (i.e. n=102) out of a total of 342 conditioned MEPs. The magnitude of each of the significant 102 facilitatory effects was multiplied by 0.30, the total incidence of significant facilitation from iSMA with all ISIs, and these values were averaged.

Statistics

The incidence of modulatory effects induced by iSMA or cSMA across ISIs were compared using Cochran's Q tests followed by post-hoc McNemar's tests. The magnitude of modulatory effects induced by iSMA or cSMA across ISIs were compared using linear mixed models with ISI as a fixed factor followed by post-hoc pairwise t-test analyses using Fisher's Least Significant Difference (LSD) correction method. Post-hoc results of McNemar's tests and pairwise t-test analyses are presented in Table 1. The incidence of modulatory effects induced by iSMA and cSMA were compared using chi-square tests followed by post hoc two-proportion Z tests. The magnitude of modulatory effects induced by iSMA and cSMA were compared using two-sample t-tests. The global impact score induced by SMA, PMd and PMv were compared using Kruskal-Wallis tests followed by Tukey-Kramer post hoc tests. Effects were considered statistically significant with p values < 0.05. When applicable, results are expressed as mean ± standard error (SE). Statistical analyses were performed using MatLab (Version R2014a; Nantick, MA, USA) and IBM SPSS Statistics (Version 25.0; IBM, Armonk, NY).

Results

We conducted a total of 25 paired-pulse protocols in 2 cebus monkeys (iSMA=14; cSMA=11). For each protocol, the T-only condition evoked a significant average MEP (>3 SD above baseline; see Methods) in at least 1 and up to 6 muscles of the contralateral arm (total=111 MEPs). These MEPs were more common in the APB (n=24), FPB (n=23), EDC (n=20) and ECU (n=18) and less common in FDS (n=15) and PL (n=11) (iSMA=total of 57 MEPs; cSMA=total of 54 MEPs). As we specifically placed our C_{stim} and T_{stim} electrodes at cortical sites evoking MEPs in digit or forearm muscles with ICMS trains, we found very few significant MEPs in proximal arm muscles (BB=5 and TB=1). These few MEPs were thus excluded from further analyses. Accordingly, our study focuses on the interactions between the outputs of M1, iSMA and cSMA involved in distal forelimb movements. All intrinsic hand and forearm MEPs collected in our study are presented as an intensity plot in Figure 4.2. The plot shows the MEPs under the 8 conditions (T-only, C-only and the 6 paired-pulse conditions). In this figure, the MEPs obtained with the C-only and the 6 paired-pulse conditions are normalized to the peak value of the MEPs obtained with the T-only condition (color scale). Following iSMA conditioning (Figure 4.2A), we found comparable cases of facilitation and inhibition, in both intrinsic hand and forearm MEPs. Following cSMA conditioning however (Figure 4.2B), inhibitory effects appeared to be more common than facilitation, once again in both intrinsic hand and forearm MEPs. When comparing the modulation of intrinsic hand and forearm MEPs for both iSMA and cSMA, no obvious differences were observed. We thus chose to pool data from the two muscle groups in further analyses.

Modulatory effects of iSMA and cSMA on M1 outputs with each ISI tested

We studied the incidence and magnitude (Z-scores; see Methods) of facilitatory and inhibitory effects induced by iSMA and cSMA conditioning on M1 outputs with each ISI we tested. For protocols in which the C_{stim} was applied in iSMA, out of the 342 MEPs (57 significant responses with T_{only} conditioned with 6 ISIs), we found 202 cases in which paired-pulse trials significantly modulated M1 outputs (59.2%). Among these significant modulations, we found 102 cases of facilitation (29.8%) and 100 cases of inhibition (29.2%). The incidence and magnitude of these two types of modulation induced by iSMA with each ISI are respectively shown in the left and right panels of Figure 4.3A. Facilitatory effects occurred with all tested ISIs but were most commonly

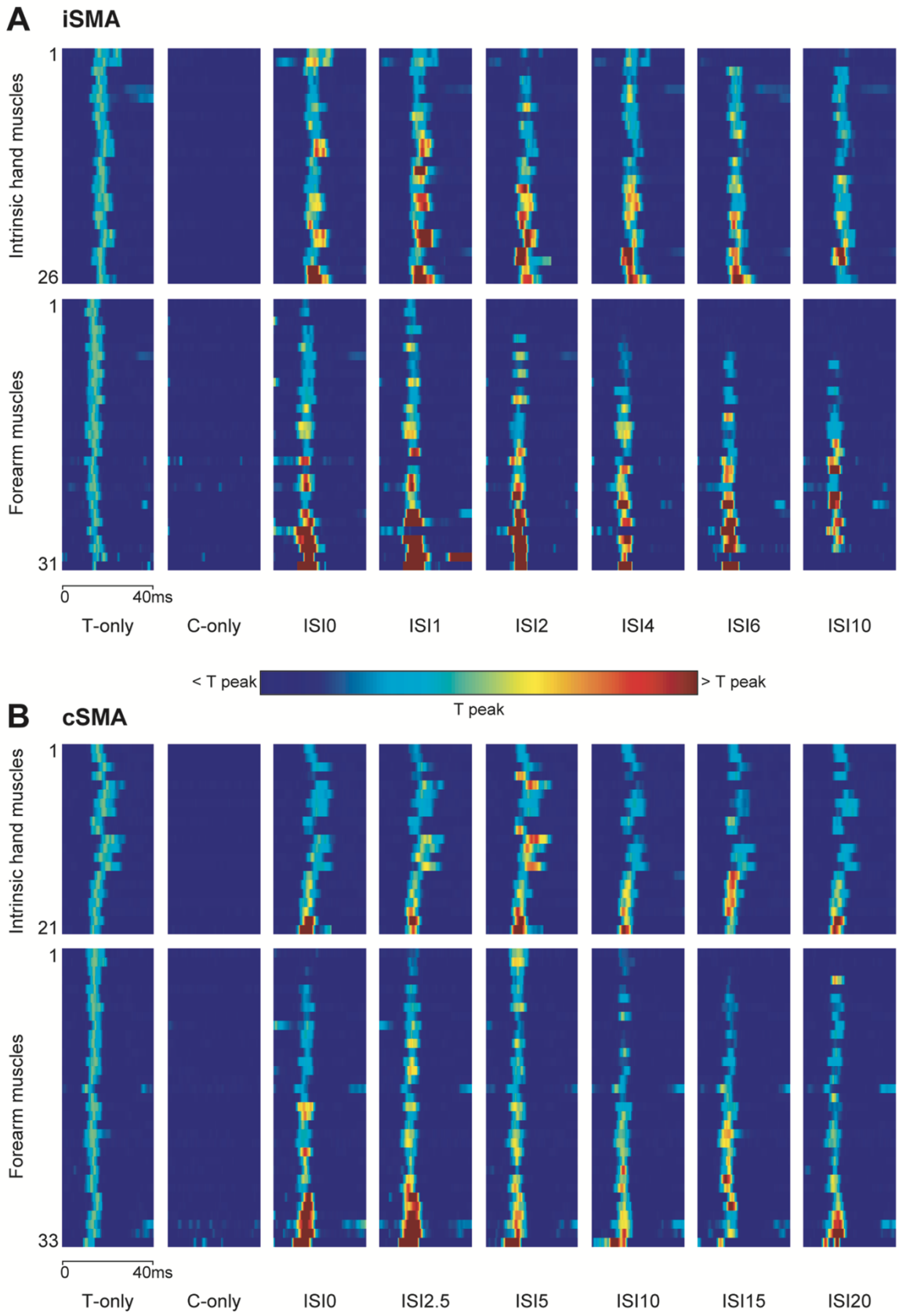

A
iSMA
Intrinsic hand muscles
1
26
Forearm muscles
1
31
0
40ms
T-only
C-only
ISI0
ISI1
ISI2
ISI4
ISI6
ISI10
< T peak
> T peak
T peak
B
cSMA
Intrinsic hand muscles
1
21
Forearm muscles
1
33
0
40ms
T-only
C-only
ISI0
ISI2.5
ISI5
ISI10
ISI15
ISI20

Figure 4.2 *Complete dataset of modulatory effects induced by iSMA and cSMA on M1 outputs*

A) Intensity plot showing the effects of iSMA conditioning on the 26 MEPs recorded in intrinsic hand muscles (FPB, APB; top panel) and on the 31 MEPs recorded in forearm muscles (ECU, EDC, PL, FDS; bottom panel). The columns, from left to right, show the responses evoked in the T-only condition, the C-only condition and the 6 different paired-pulse conditions (ISI0, ISI1, ISI2, ISI4, ISI6 and ISI10). In this plot, responses in the C-only and paired-pulse conditions are normalized to the peak value of the MEP obtained in the T-only condition (color scale below). The rows, from top to bottom, are individual MEPs ordered based on their mean peak amplitude across ISIs, from lowest to largest. In the C-only condition, no clear responses are observed as the C_{stim} was set to a sub-threshold value. In paired-pulse conditions, there was no clear differences in the incidence of facilitation and inhibition on the MEPs when comparing intrinsic hand and forearm muscles. **B)** Effects of cSMA conditioning on the 21 MEPs recorded in intrinsic hand muscles (top panel) and on the 33 MEPs recorded in forearm muscles (bottom panel). Apart from the different ISIs used for paired-pulse conditions (ISI0, ISI2.5, ISI5, ISI10, ISI15 and ISI20), the columns and rows are as in A. Once again no obvious differences in the incidence of inhibition and facilitation were observed when comparing intrinsic hand and forearm muscles.

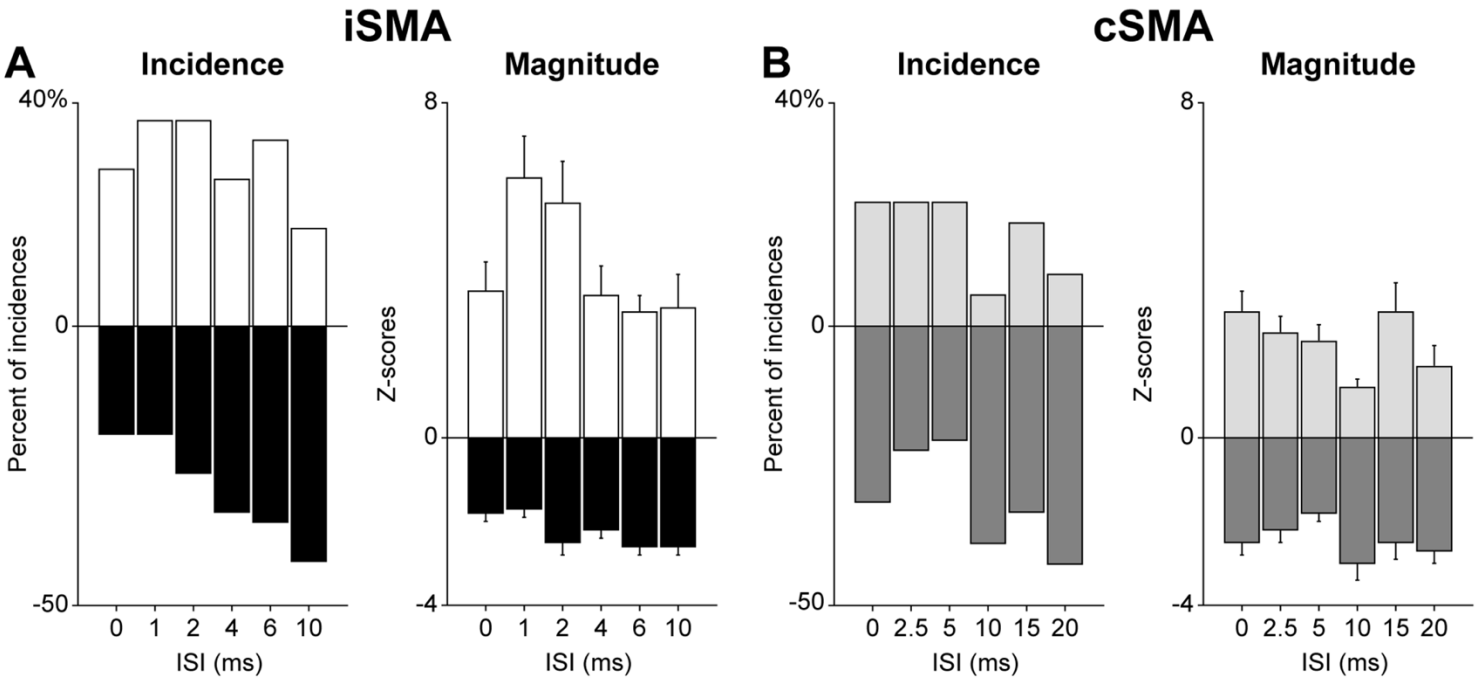


Figure 4.3 *Modulations of iSMA and cSMA on the outputs of M1 with each ISI tested*

A) Incidence (left panel) and magnitude (right panel) of modulatory effects induced by iSMA with each tested ISI. In the left panel, each bar represents the percentage of the 57 MEPs that were significantly modulated with a given ISI. For example, with ISI0 (i.e. simultaneous delivery of the C_{stim} and T_{stim}), 16 of the 57 MEPs (28.1%) had a significant facilitatory effect and 11 (19.3%) had a significant inhibitory effect on the outputs of M1. In the right panel, each bar represents the mean (± SE) of positive and negative *Z*-scores obtained with each ISI. Facilitatory effects were more common and powerful with short ISIs while inhibitory effects more common and slightly more powerful with longer ISIs. **B)** Incidence (right panel) and magnitude (left panel) of modulatory effects induced by cSMA on M1 outputs with each tested ISI. As for iSMA, cSMA tended to induce more facilitatory effects with short ISIs and more inhibitory effects with longer ISIs.

found when the C_{stim} preceded the T_{stim} by 1 or 2ms (ISI1 n=21 and ISI2 n=21). With all tested ISIs, we also found cases in which the conditioning of iSMA induced significant inhibitory effects. These inhibitory effects were most likely to occur with longer ISIs, especially when the C_{stim} preceded the T_{stim} by 10ms (ISI10 n=24). When we statistically compared the incidences of facilitatory and inhibitory effects induced by iSMA across ISIs with Cochran's Q tests, we found that the incidence of facilitatory and inhibitory effects depended on the ISI (facilitation: p=0.039; inhibition: p=0.007). Post-hoc McNemar's tests (Table 1) showed that the incidence of facilitation was significantly greater with ISI1, ISI2 and ISI6 in comparison to ISI10 (p=0.035; p=0.019; p=0.022) and that the incidence of inhibition was significantly greater with ISI10 than with ISI0 (p=0.011) and ISI1 (p=0.011). The magnitude of modulation followed a pattern similar to the one described for incidence. Facilitatory effects were more powerful with ISI1 and ISI2 and although the strength of inhibitory effects varied less with the different ISIs, they also tended to be more powerful with longer ISIs (ISI6 and ISI10). To statistically compare the magnitude of facilitatory or inhibitory effects induced by iSMA across ISIs, we used linear mixed models with ISI as a fixed factor. The intercept-only model indicated that the use of mixed models was warranted to compare the magnitude of both facilitation (Intraclass correlation (ICC)=0.239; p=0.006) and inhibition (ICC=0.167; p=0.023) across ISIs. The ISI fixed effect in both facilitation and inhibition mixed models was significant (facilitation: p<0.001; inhibition: p=0.017). Post-hoc pairwise t-test analyses (Table 1) showed that both ISI1 and ISI2 had significantly more powerful facilitatory effects compared to ISI4 (p<0.001; p=0.009), ISI6 (p<0.001; p=0.003) and ISI10 (p<0.001; p=0.008). In addition, ISI1 induced significantly more powerful facilitation compared to ISI0 (p=0.002). For inhibition, both ISI6 and ISI10 had significantly more powerful inhibitory effects than ISI0 (p=0.028; p=0.014) and ISI1 (p=0.008; p=0.003). In addition, ISI2 induced significantly more powerful inhibition compared to ISI1 (p=0.024). Thus, facilitatory effects occurring with ISI1 and ISI2 and inhibitory effects occurring with ISI10 were not only more frequent (Figure 4.3A, left panel), they were also more powerful (Figure 4.3A, right panel).

When cSMA was the source of conditioning stimuli, out of the 324 MEPs (54 significant responses with T_{only} conditioned with 6 ISIs), we found 156 cases in which cSMA significantly modulated M1 outputs (48.1%). Across these significant modulations, we found fewer cases of facilitation (54 cases; 16.7%) than inhibition (102; 31.5%). The incidence and magnitude of these significant facilitatory and inhibitory effects with each ISI are shown in Figure 4.3B. As for iSMA,

		P Values for Incidences (McNemar's Test)		*P* Values for Magnitude (Pairwise *t* Test Analyses)	
		Facilitation	Inhibition	Facilitation	Inhibition
iSMA	ISI0 vs ISI1	0.125	1.00	0.002*	0.670
	ISI0 vs ISI2	0.302	0.424	0.053	0.065
	ISI0 vs ISI4	1.00	0.077	0.464	0.310
	ISI0 vs ISI6	0.629	0.078	0.259	0.028*
	ISI0 vs. ISI10	0.263	0.011*	0.346	0.014*
	ISI1 vs. ISI2	1.00	0.481	0.220	0.024*
	ISI1 vs. ISI4	0.263	0.057	0.000*	0.147
	ISI1 vs. ISI6	0.824	0.078	0.000*	0.008*
	ISI1 vs. ISI10	0.035*	0.011*	0.000*	0.003*
	ISI2 vs. ISI4	0.146	0.481	0.009*	0.393
	ISI2 vs. ISI6	0.791	0.359	0.003*	0.732
	ISI2 vs. ISI10	0.019*	0.078	0.008*	0.579
	ISI4 vs. ISI6	0.344	1.00	0.678	0.223
	ISI4 vs. ISI10	0.180	0.332	0.766	0.141
	ISI6 vs. ISI10	0.022*	0.344	0.929	0.835
cSMA	ISI0 vs ISI2.5	1.00	0.180	0.480	0.358
	ISI0 vs ISI5	1.00	0.210	0.268	0.134
	ISI0 vs ISI10	0.012*	0.424	0.010*	0.081
	ISI0 vs ISI15	0.815	1.00	0.695	0.606
	ISI0 vs. ISI20	0.039*	0.210	0.052	0.427
	ISI2.5 vs. ISI5	1.00	1.00	0.662	0.551
	ISI2.5 vs. ISI10	0.022*	0.049*	0.035*	0.013*
	ISI2.5 vs. ISI15	0.791	0.146	0.278	0.162
	ISI2.5 vs. ISI20	0.039*	0.013*	0.158	0.100
	ISI5 vs. ISI10	0.012*	0.031*	0.074	0.003*
	ISI5 vs. ISI15	0.815	0.092	0.144	0.053
	ISI5 vs. ISI20	0.039*	0.012*	0.291	0.031*
	ISI10 vs. ISI15	0.039*	0.581	0.004*	0.203
	ISI10 vs. ISI20	0.687	0.804	0.491	0.338
	ISI15 vs. ISI20	0.267	0.267	0.025*	0.772

Table 1. *Results of post hoc tests comparing the incidence and magnitude of facilitatory and inhibitory effects across ISIs for iSMA and cSMA.*

ISI, interstimulus interval (in ms); iSMA and cSMA, ipsilateral and contralateral supplementary motor area, respectively. *P < 0.05.

facilitatory effects were more frequent with shorter ISIs (ISI0 n=12, ISI2.5 n=12, ISI5 n=12) and inhibitory effects with longer ISIs (ISI10 n=21, ISI15 n=18 and ISI20 n=23). When we statistically compared the incidences of facilitatory and inhibitory effects induced by cSMA across ISIs, we found that the incidence of facilitatory and inhibitory effects depended on the ISI (facilitation: p=0.019; inhibition: p=0.008). Post-hoc tests (Table 1) showed that the incidence of facilitation was greater with ISI0, ISI2.5, ISI5 in comparison to ISI10 (p=0.012; p=0.022; p=0.012, respectively) and ISI20 (p=0.039; p=0.039; p=0.039, respectively). Moreover, the incidence of facilitation was greater with ISI15 than with ISI10 (p=0.039). For the incidence of inhibition, it was greater with ISI10 and ISI20 than with ISI2.5 (p=0.049; p=0.013) and ISI5 (p=0.031; p=0.012). Once again, the magnitude of modulation followed a pattern that was quite similar to that of incidence. The intercept-only model indicated that the use of mixed models was warranted to compare the magnitude of both facilitatory (ICC=0.180; p=0.043) and inhibitory (ICC=0.407; p=0.001) effects across ISIs. The ISI fixed effect in both facilitation and inhibition mixed models was significant (facilitation: p=0.031; inhibition: p=0.040). Post-hoc analyses (Table 1) showed that ISI0, ISI2.5 and ISI15 had more powerful facilitatory effects than ISI10 (p=0.010; p=0.035; p=0.004) and that ISI15 induced significantly more powerful facilitation than ISI20 (p=0.025). For inhibition, both ISI10 and ISI20 had significantly more powerful inhibitory effects compared to ISI5 (p=0.003; p=0.031) and ISI10 induced significantly more powerful inhibition than ISI2.5 (p=0.013).

Next, to statistically compare the incidence of effects induced by iSMA and cSMA, we pooled data from all tested ISIs and used a chi-square test followed by post-hoc two-proportion Z tests. The pattern of modulation of iSMA was different from that of cSMA (χ^2=16.86; p<0.001). Specifically, iSMA induced significantly more facilitatory effects (29.8%) (p<0.001) than its contralateral counterpart (16.7%). However, both areas induced a similar proportion of inhibitory effects (29.2% and 31.5%, respectively) (p=0.53) (Figure 4.4A). We also compared the magnitude of the modulatory effects induced by iSMA and cSMA. A first two-sample t-test comparing facilitatory effects showed that iSMA induced more powerful facilitatory effects than cSMA (mean Z-scores=4.20 and 2.30; t=4.53; p<0.001) and another two-sample t-test comparing inhibitory effects showed no significant differences between iSMA and cSMA (mean Z-scores=-2.25 and -2.51; t=1.49; p=0.14) (Figure 4.4B).

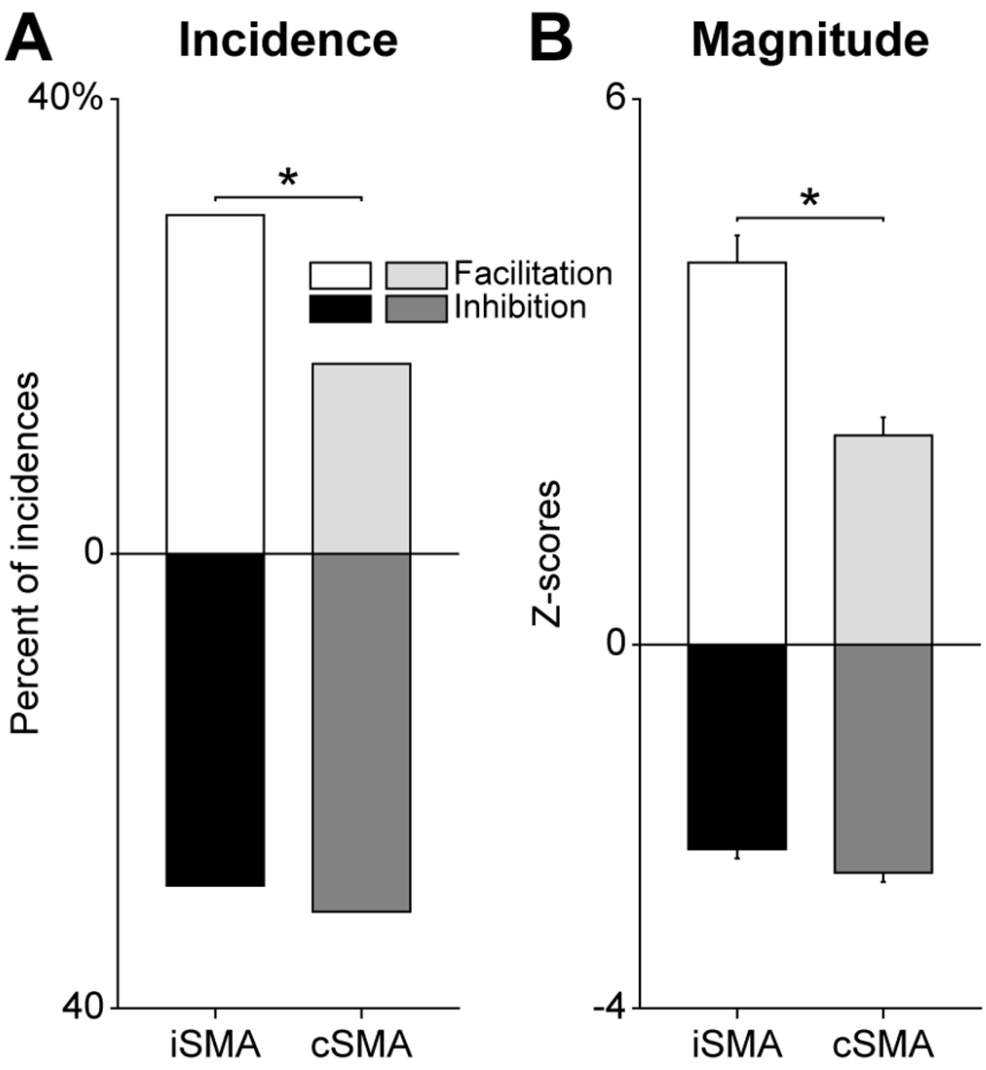


Figure 4.4 *Comparison of the modulatory effects of iSMA and cSMA with all tested ISIs pooled*

A) Incidence of facilitatory and inhibitory effects induced by iSMA and cSMA. Facilitatory effects were significantly more frequent following iSMA compared to cSMA conditioning (chi-square test). **B)** Magnitude of facilitatory and inhibitory effects induced by iSMA and cSMA. Facilitatory effects were significantly more powerful following iSMA compared to cSMA conditioning (two-sample t-test). * $p<0.05$.

Patterns of modulatory effects of iSMA and cSMA across ISIs

We then analyzed how individual MEPs evoked with T-only trials were modulated across ISIs and whether there were differences between those modulated by iSMA (n=57) and cSMA (n=54). To do so, MEPs evoked with T-only trials were classified into 3 groups (Deffeyes et al., 2015). First, the conditioning of iSMA or cSMA could significantly facilitate the MEP with at least one ISI, but never significantly inhibit the MEP with any of the other ISIs (i.e. pure facilitation across ISIs). Second, the conditioning of iSMA or cSMA could significantly inhibit the MEP with at least one ISI, but never significantly facilitate the MEP with any of the other ISIs (i.e. pure inhibition across ISIs). Third, the conditioning of iSMA or cSMA could significantly facilitate the MEP with at least one ISI and also significantly inhibit the MEP with at least one ISI (i.e. opposite effects across ISIs; see for example the case shown in Figure 4.1E-F). We found very few MEPs that were not modulated with any of the ISIs (iSMA n=0; cSMA n=3), suggesting that iSMA and cSMA were very likely to modulate the outputs of M1 to intrinsic hand and forearm muscles with the ISIs we tested (100.0% and 94.4% for iSMA and cSMA, respectively).

Out of the population of 57 MEPs modulated by iSMA, we found slightly fewer pure facilitatory (19 cases, 33.3%) than pure inhibitory effects (23 cases, 40.4%) and we found 15 cases of opposite effects (26.3%) (Figure 4.5A). For cSMA, out of the population of 54 MEPs, we again found slightly fewer pure facilitatory (18 cases, 33.3%) than pure inhibitory effects (24 cases, 44.4%) and we found 9 cases of opposite effects (16.7%). The patterns of effects across ISIs of iSMA and cSMA were not significantly different (χ^2=1.30; p=1.04). Hence, it appears that equal proportions of cortical territory within iSMA and cSMA induce pure facilitatory, pure inhibitory and opposite effects across ISIs.

Modulatory effects of iSMA and cSMA conditioning across recorded muscles

We then inspected how a conditioning stimulation simultaneously modulated the various MEPs across the recorded muscles and whether there were differences between iSMA and cSMA. To do so, the MEPs modulated by the same paired-pulse protocol (i.e. cortical stimulation sites) were pooled together for each ISI (14 protocols x 6 ISI=84 cases for iSMA; 11 protocols x 6 ISI=66

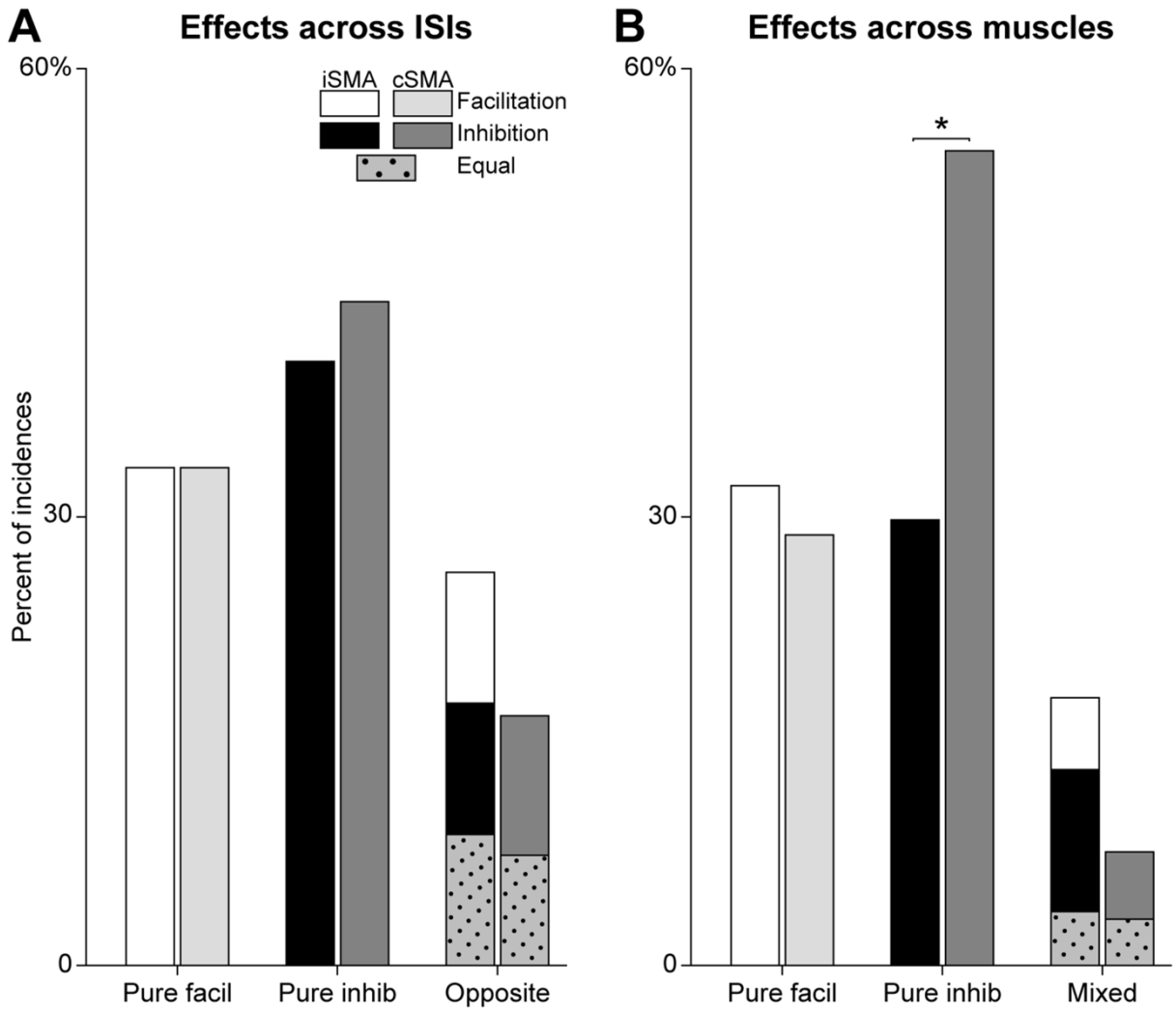


Figure 4.5 *Groups of modulatory effects across ISIs and across muscles for iSMA and cSMA*

A) Incidence of pure facilitatory, pure inhibitory, and opposite effects across ISIs for iSMA and cSMA. The incidence of opposite effects is further divided into cases where MEPs were predominantly facilitated (i.e. more ISIs with facilitation than ISIs with inhibition; white), predominantly inhibited (i.e. more ISIs with inhibition than ISIs with facilitation; black) or equally facilitated and inhibited across ISIs (same number of ISIs with facilitation and inhibition; dotted pattern). The patterns of effects across ISIs induced by iSMA and cSMA were not significantly different (chi-square test). **B)** Incidence of pure facilitatory, pure inhibitory, and mixed effects across muscles for iSMA and cSMA. Here again, the incidence of mixed effects is further divided into cases where MEPs across muscles were predominantly facilitated (i.e. more muscles with facilitation than muscles with inhibition), predominantly inhibited (i.e. more muscles with

inhibition than muscles with facilitation) or equal numbers of muscles simultaneously facilitated and inhibited (same number of muscles with facilitation and inhibition; dotted pattern). Conditioning stimuli in iSMA induced significantly less pure inhibition across muscles than in cSMA (chi-square test). * $p<0.05$.

cases for cSMA). Once again, we classified the modulatory effects into 3 groups (Deffeyes et al., 2015). With a given ISI, the conditioning stimulation at a cortical site could simultaneously facilitate the MEPs of all modulated muscles (i.e. pure facilitation across muscles), could inhibit the MEPs of all modulated muscles (i.e. pure inhibition across muscles) or could simultaneously facilitate and inhibit different combinations of muscles (i.e. simultaneous mixed effects across recorded muscles).

Out of the cases in which the conditioning stimulation was delivered in iSMA, we found 27 cases of pure facilitation (32.1%) and in 24 of these (88.9%), more than one muscle was simultaneously facilitated (Figure 4.5B). We found 25 cases of pure inhibition (29.8%) and in 18 of these (72.0%), more than one muscle was simultaneously inhibited. Finally, there were 15 cases of simultaneous mixed effects (17.9%). When the conditioning stimulation was in cSMA, we found 19 cases of pure facilitation (28.8%) and in 10 of these cases (52.6%) the effect was observed in more than one muscle. There were many more cases of pure inhibition (n=36, 54.5%) and in 26 of these cases (72.2%), more than one muscle was simultaneously inhibited. Finally, there were 5 cases of simultaneous mixed effects (7.6%). Using a chi-square test, we found that the pattern of effects across muscles depended on whether the conditioning stimulation was delivered in iSMA or cSMA (χ^2=8.65; p=0.03) (Figure 4.5B). However, post-hoc two-proportion Z-tests revealed that only pure inhibitory effects differed, with iSMA inducing significantly less of these effects than cSMA (p=0.002). In the case of pure facilitatory effects and simultaneous mixed effects, the incidence was the same whether the conditioning was in iSMA or cSMA (p=0.66 and p=0.07, respectively). Overall, both iSMA and cSMA predominantly evoked consistent effects across the muscle field targeted by the outputs of M1 (pure facilitation and pure inhibition) rather than mixed effects across the muscles we recorded.

Comparing the modulatory impacts of SMA to those of PMd and PMv

To compare the modulatory patterns of SMA to those of PMd and PMv collected in the same series of experiments (Quessy et al., 2016; Côté et al., 2017), we combined the incidence and magnitude of modulatory effects from each premotor area into a single impact score (see methods). Figure 4.6 shows the impact scores for all 3 premotor areas with each ISI we tested. The strength of modulatory impacts originating from all premotor areas depended on the timing between the C_{stim}

and T_{stim} (ISIs). However, the ISIs with the strongest impact scores often differed when comparing SMA, PMd and PMv. For example, in the ipsilateral hemisphere (Figure 4.6A), iPMd had a much greater facilitatory impact on the production of motor outputs than either iSMA or iPMv when the C_{stim} and T_{stim} were delivered simultaneously (ISI0). In contrast, when the conditioning stimuli were delivered 4, 6 or 10ms prior to M1 (ISI4, ISI6 and ISI10), iPMv had a much stronger facilitatory impact than iPMd or iSMA. With short ISIs (ISI1 and ISI2), all 3 premotor areas had comparably strong facilitatory impact values. For ipsilateral inhibitory effects, iPMd had the strongest inhibitory impact of all 3 premotor areas, regardless of the ISI. The inhibitory impact of iPMd was greatest when the C_{stim} was delivered 6ms prior to the T_{stim} in M1 (ISI6) while iSMA and iPMv had their greatest inhibitory impact with ISI10.

In the contralateral hemisphere (Figure 4.6B), cPMd and cPMv respectively had the strongest facilitatory and inhibitory impact values, regardless of the ISI tested. The facilitatory impact of cPMd was strongest with ISI15, but was also strong with ISI0, ISI5 and ISI10. The greatest facilitatory impact from cSMA and cPMv were with ISI5. For inhibition from contralateral premotor areas, cPMv had its strongest inhibitory impact with long ISIs (ISI15 and ISI20) while the greatest inhibitory impact induced by both cSMA and cPMd was with ISI10. In conclusion, regardless of the ISI, the nature of the modulation (i.e. facilitation or inhibition) or the hemisphere tested, the impact of SMA never had the highest value among premotor areas. Rather, its impact on M1 outputs was either the weakest or lied between the values of PMd and PMv.

For each premotor area, we then calculated the global impact score (i.e. impact across ISIs, see methods) of each premotor area for both facilitatory and inhibitory effects. The global impact scores of ipsilateral and contralateral SMA, PMd and PMv on M1 outputs are presented in a 'box and arrow' diagram in Figure 4.7. Each premotor area had a unique pattern of global impact scores. For intrahemispheric modulations, iPMv had the strongest facilitatory impact and iPMd the strongest inhibitory impact (3.5 ± 0.46 and -1.9 ± 0.07, respectively). To compare the strength of the impact between each premotor area we used Kruskal-Wallis tests. We found that the strength of both facilitatory and inhibitory impacts was dependent on the premotor area (facilitation: $H=14.35$; $p=0.001$, inhibition: $H=139.20$; $p<0.001$). Post-hoc Tukey-Kramer tests showed that iPMd and iPMv had a stronger facilitatory impact than iSMA ($p=0.01$ and $p=0.002$) and that iPMd had a stronger inhibitory impact than iSMA ($p<0.001$) and iPMv ($p<0.001$). Finally, iSMA had a

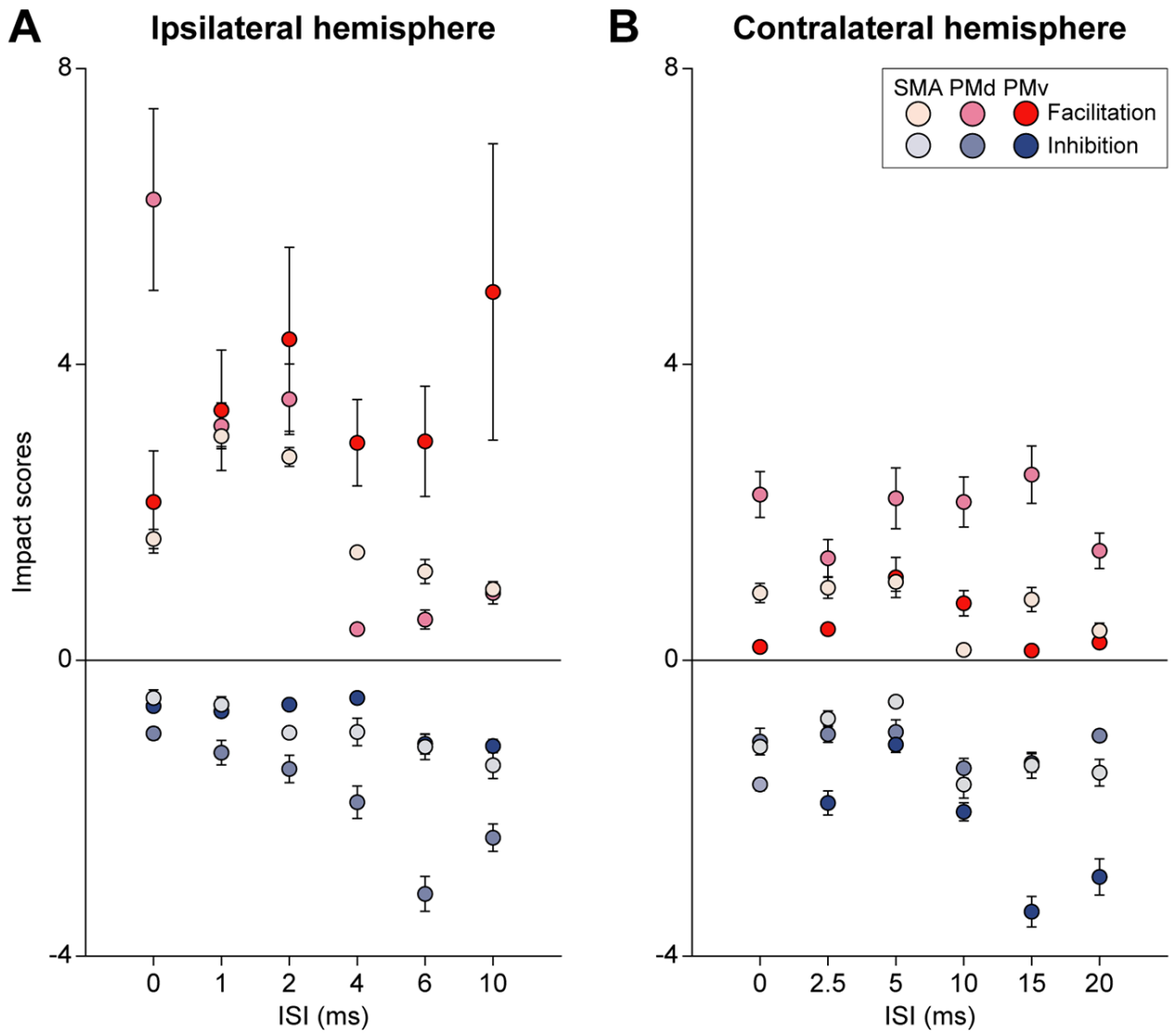


Figure 4.6 *Impact scores of SMA, PMd and PMv on M1 outputs with each ISI*

A) Facilitatory and inhibitory impacts of ipsilateral SMA, PMd and PMv with each ISI we tested ± SE. Overall, the strongest facilitatory impact was with ISI0 when iPMd was the source of conditioning. With longer ISIs, the strongest facilitatory impact was from iPMv (ISI4, ISI6 and ISI10). Finally, all 3 premotor areas had strong facilitatory impacts with ISI1 and ISI2. For inhibition, iPMd had the strongest impact regardless of the ISI, and it exerted its strongest inhibitory impact with ISI6. For iSMA and iPMv, the strongest inhibitory impact was with ISI10 and ISI6, respectively. **B)** Facilitatory and inhibitory impacts of contralateral SMA, PMd and PMv with each ISI. Among premotor areas, cPMd had the strongest facilitatory impact regardless of the ISI, and its strongest impact occurred with ISI15. The strongest facilitatory impacts of cSMA and

cPMv were with ISI5. For inhibition, cPMv had the strongest impact regardless of the ISI, with its strongest impact occurring with ISI15. For both cSMA and cPMv, the strongest inhibitory impact was with ISI10. Overall, regardless of the ISI, the nature of the modulation or the hemisphere, SMA never had the highest impact value among all 3 premotor areas.

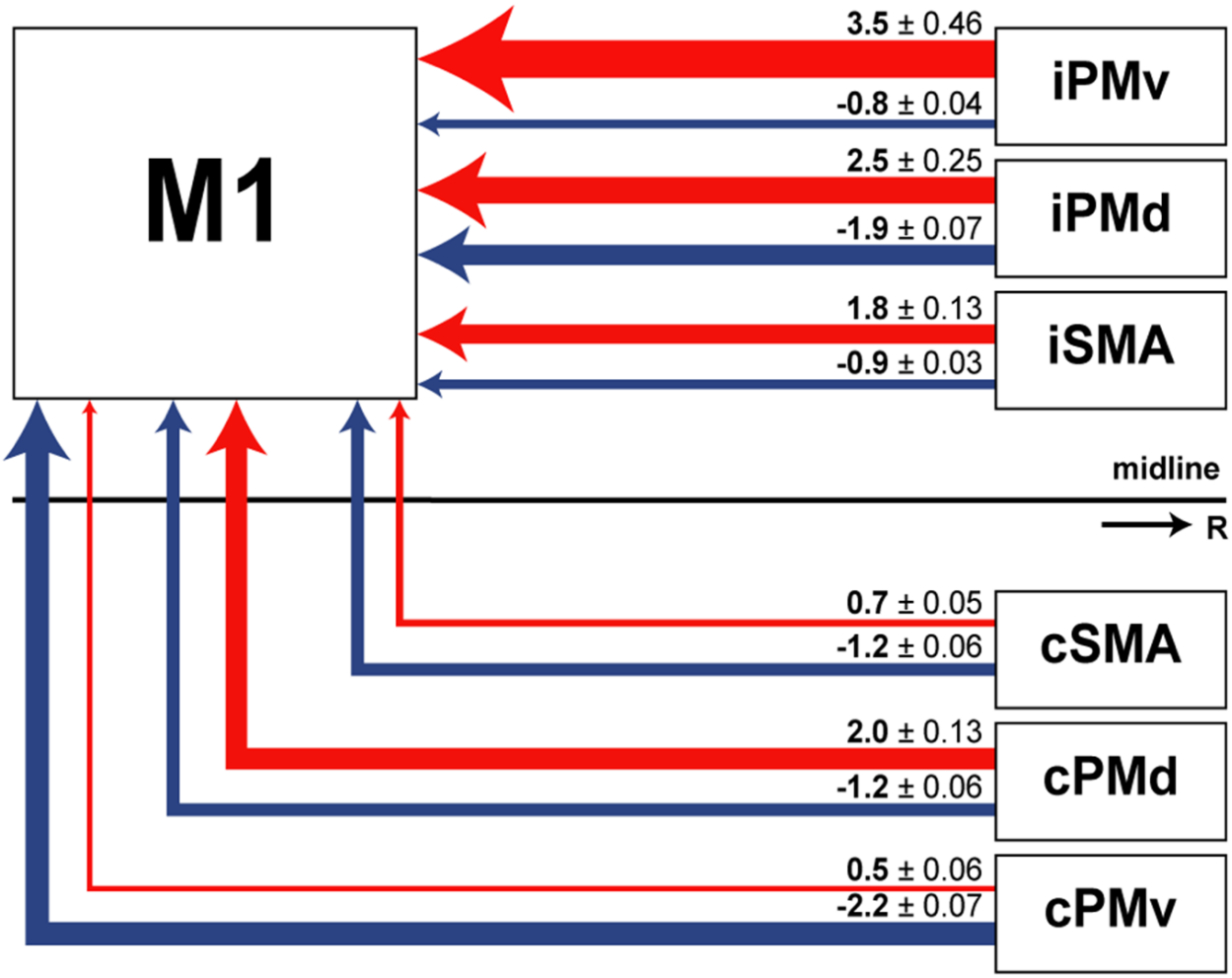


Figure 4.7 *Global impact of SMA, PMd and PMv modulations on M1 outputs*

Summary of the global facilitatory and inhibitory impacts of SMA, PMd and PMv of both hemispheres ± SE. The thickness of the arrows is proportional to the intensity of the impact and the global impact values are shown at the origins of the arrows. When comparing the impact scores of premotor areas within each hemisphere, unique patterns of modulation are observed. In the ipsilateral hemisphere, iPMv had the strongest facilitatory impact and iPMd had the strongest inhibitory impact. The opposite pattern is present in the contralateral hemisphere. The strongest inhibitory impact on M1 outputs is induced by cPMv and the strongest facilitatory impact is induced by cPMd. Consequently, in both hemispheres, SMA consistently has a subtler modulatory impact in comparison to either PMd or PMv. R: rostral.

stronger inhibitory impact than iPMv ($p=0.02$). For interhemispheric modulations, cPMd had the strongest facilitatory impact and cPMv the strongest inhibitory impact (2.0 ± 0.13 and -2.2 ± 0.07). Using Kruskal-Wallis tests, we again found that the strength of both facilitatory and inhibitory impacts was dependent on the premotor area (facilitation: $H=102.71$; $p<0.001$, inhibition: $H=120.71$; $p<0.001$). Post-hoc Tukey-Kramer tests showed that cPMd had a stronger facilitatory impact than both cSMA ($p<0.001$) and cPMv ($p<0.001$) and that cPMv had a stronger inhibitory impact compared to both cSMA ($p<0.001$) and cPMd ($p<0.001$). Overall, although PMd and PMv had highly contrasting patterns of modulation (Côté et al., 2017), SMA appeared to fall more in the middle. As for impact values with the various ISIs, the global impact value for SMA was never the strongest among premotor areas, regardless of the nature of the modulation or the hemisphere.

Discussion

Isolating stimulation to the supplementary motor area (SMA) of a given hemisphere in humans using TMS is challenging. In the present study, we unequivocally isolated conditioning stimulations to either iSMA or cSMA using invasive paired-pulse protocols with ICMS in cebus monkeys. This approach allowed us to compare their respective modulatory effects on the outputs of M1. We found clear similarities in the pattern of modulation from the two areas. In particular, facilitatory effects were more frequent with short ISIs and inhibitory effects more frequent with long ISIs for both iSMA and cSMA. There were also some significant differences. Namely, facilitatory effects from iSMA were more frequently evoked and were more powerful than from cSMA. When comparing the pattern of modulation of SMA to other premotor areas, we found that the impact of SMA on M1 outputs was always weaker than the one of either PMd or PMv, regardless of the hemisphere, the nature of the modulation (i.e. facilitation or inhibition) or the ISI tested. Together, our results demonstrate that premotor areas in the ipsi and contralateral hemisphere have specific and unique patterns of modulation that could support their distinct functions for the production of hand movements.

Modulatory effects of iSMA and cSMA on M1 outputs

Paired-pulse TMS experiments in humans have been at the forefront of research on the 'physiological connectivity' between premotor areas and M1 (Civardi et al., 2001; Koch et al., 2006; Davare et al., 2008; Arai et al., 2012). Whereas TMS possesses various advantages, one drawback is that isolating the stimulation in one hemisphere to distinguish the modulatory effects of iSMA and cSMA is challenging (Fiori et al., 2017). Using ICMS enabled us to place electrodes deeper along the medial wall and use small current intensities in order to disentangle the modulatory effects induced by the two areas. Interestingly, this revealed clear similarities in their patterns of modulation. This contrasts with PMd and PMv, for which modulatory effects with the various ISIs we tested are quite different for the ipsi and contralateral hemisphere (Quessy et al., 2016; Côté et al., 2017). In the case of PMd and PMv, it is possible that these unique patterns of modulation predispose the ipsi and contralateral counterparts to undertake opposing roles at different stages of the production of motor outputs. In contrast, the similarities between the patterns of modulation originating from iSMA and cSMA may allow them to work more synergistically to

control movement sequences, for instance during bimanual tasks (Tanji, 1994; Sadato et al., 1997). Given the common pattern of modulation of iSMA and cSMA with the different ISIs, another possibility is that both SMAs synergistically modulate motor outputs in function of the urgency to produce motor outputs. For instance, bihemispheric facilitation with short ISIs could be particularly useful in high urgency situations to produce rapid motor responses while inhibitory effects with longer ISIs may be more useful in low urgency situations to prepare precise motor responses.

Comparison of iSMA and cSMA also showed that the incidence and strength of facilitatory effects from iSMA are greater than from cSMA. This is in line with our findings for PMd and PMv. For both these areas, ipsilateral facilitatory effects on M1 outputs are greater than those of the opposite hemisphere (Quessy et al., 2016; Côté et al., 2017). It is well documented that premotor areas have more intra than interhemispheric connections with M1 (Rouiller et al., 1994; Dancause et al., 2006b; Dancause et al., 2007). These numerous connections provide a potential pathway to support powerful ipsilateral facilitatory effects. Along these lines, we found that facilitation from iSMA are most powerful when the C_{stim} is delivered 1 or 2ms prior to the T_{stim}, which would allow time for the interaction to take place at the cortical level, much like what has been proposed for PMv (Cerri et al., 2003; Shimazu et al., 2004).

Finally, the use of ICMS allowed us to stimulate small cortical territories within the hand representation of iSMA and cSMA. Assuming a k value of 1.292, the radius of directly stimulated cortex by the C_{stim} of 225μA can be estimated to ~0.4mm (Stoney et al., 1968). This revealed that subpopulations of neurons within SMA have a broad range of modulatory effects from facilitatory to inhibitory. In keeping with this finding, studies in macaque monkeys have shown that ICMS delivered in iSMA can both excite or inhibit M1 neurons (Aizawa and Tanji, 1994; Tokuno and Nambu, 2000). Together, results obtained with ICMS in primates are consistent with those of TMS studies in humans demonstrating that stimulation of SMA can facilitate as well as inhibit M1 outputs to hand muscles depending, among other parameters, on the timing between the two stimulations (i.e. ISIs) (Oliveri et al., 2003; Arai et al., 2012; Fiori et al., 2017). Our paired-pulse experiments in the sedated monkey provides information about the range of potential effects that premotor areas can have on the outputs of M1 (Côté et al., 2017). In the awake state, the variability we observed in the modulations induced by SMA may allow it to contribute to multiple aspects of motor actions. Specific subpopulations of neurons within SMA might be preferentially activated

so as to shift the balance towards facilitation or inhibition during different stages of movement or depending on task demands.

Modulatory impact of SMA in comparison to PMd and PMv

When comparing the three premotor areas, one striking finding is that SMA never had the greatest facilitatory or inhibitory impact on M1 outputs, regardless of the hemisphere or even the ISI tested. The more subtle impact SMA on M1 outputs could be explained by the sparser intra and interhemispheric projections from SMA to M1, in comparison to either PMd or PMv (Rouiller et al., 1994; Dum and Strick, 2005; Dea et al., 2016). It has also been shown that SMA sends more intrahemispheric projections to the hand representation of PMd and PMv than to the hand representation of M1 in cebus monkeys (Dum and Strick, 2005). One possibility is that among premotor areas, SMA may be more involved in the regulation and monitoring of neural activity and outputs of other premotor areas than in the modulation of M1 outputs *per se*. In addition, stimulus triggered averaging studies in awake macaques have reported that stimulation in SMA evokes slower and weaker EMG responses in comparison to PMd or PMv (Boudrias et al., 2010b). This raises the possibility that while SMA has numerous corticospinal projections (He et al., 1995; Dum and Strick, 1996; Maier et al., 2002), these projections have weaker effects on hand muscles than those from PMd or PMv. Together with the present findings, these data suggest that both cortico-cortical and corticospinal projections from SMA have a more limited influence on the production of motor outputs to hand and forelimb muscles than those from PMd or PMv. Functionally, subtler modulations from SMA may be useful for refining outgoing outputs to insure the smooth progression of a movement sequence for example (Shima and Tanji, 1998, 2000), rather than entirely shifting the state of the motor system in the context of action selection (Cisek and Kalaska, 2005) or insuring flawless lateralization of outputs for the production of unimanual movements (Duque et al., 2005b; Quessy et al., 2016).

It is worth noting that, somewhat in contrast with our results, conditioning stimulations in iSMA were shown to be more likely to modulate neurons recorded in M1 (i.e. either facilitation or inhibition) in comparison to either iPMd or iPMv (Tokuno and Nambu, 2000). It is thus possible that while iSMA has weaker effects on M1 outputs in comparison to other premotor areas, it exerts a greater direct influence on M1 neurons. However, this would be surprising given the sparser

cortical projections from iSMA to M1 in comparison to both iPMd and iPMv (Dum and Strick, 2005). Alternatively, the apparent discrepancy across findings may be explained by the number of conditioning sites tested. In the Tokuno and Nambu (2000) study, conclusions relied on the investigation of the effects of a single conditioning site per premotor area per monkey (n=2). Given the great variability of modulatory effects we have found across the numerous conditioning sites that we tested in each premotor area (Quessy et al., 2016; Côté et al., 2017), their limited sampling may thus have over or underestimated the effects of any given premotor area on M1 neurons discharge pattern.

Potential implications for neuromodulatory protocols

The exhaustive comparisons of modulatory effects from diverse premotor areas we conducted could be useful to guide the development of new neuromodulatory protocols. In particular, some predictions about the effects of repetitive TMS (rTMS) on the production of motor outputs, and consequently on the production of movements, can be proposed based on the global impact scores (Figure 4.7). In the injured state such as after stroke, the objective of rTMS protocols is generally to increase the excitability of the ipsilesional M1 (Hummel and Cohen, 2006; Nowak et al., 2009). In cases in which ipsilesional premotor areas are spared by the lesion, excitatory rTMS protocols over iPMv could increase its powerful facilitatory impact on M1 and may be the most promising avenue to achieve this goal. After larger middle cerebral artery occlusions (MCAo) with damage to lateral premotor areas (i.e. iPMv and iPMd), the most effective approach may be to apply facilitatory rTMS protocols over iSMA. However, targeting contralesional premotor areas may be a better choice after such large lesions, in more severely affected patients. If so, inhibitory rTMS over cPMv to decrease its inhibitory effects on M1, or facilitatory rTMS over cPMd to favor its facilitatory impact on M1 may be better options. Consistent with this idea, excitatory rTMS over the contralesional PMd was recently reported to improve reaching in patients with severe impairments (Sankarasubramanian et al., 2017).

Nevertheless, simply aiming at increasing excitability of the ipsilesional M1 may not always be the optimal strategy. Perhaps the premotor area targeted by rTMS will have to be chosen according to the deficits of the patient and rehabilitation objectives. In such treatments, one could imagine using excitatory rTMS over SMA in combination with practice of more complex bimanual

movements or movements that require the coordination of multiple actions (Tanji, 1994; Sadato et al., 1997).

Obviously, these various predictions will have to be experimentally tested. It is not clear if and how modulatory effects from the premotor areas change after brain injury or to what degree these changes are affected by different factors such as the lesion size and location. To be most effective, the design of neuromodulatory protocols will have to take these factors into consideration and be based on a solid understanding of cortical interactions as well as post-lesion plasticity. While much work remains to be done, the large differences of modulatory impacts we found across premotor areas certainly suggest that they could be used in neuromodulatory protocols to induce a wide range of effects, and perhaps offer alternative and more effective targets in some patients that suffered brain injuries.

Chapitre 5 – Discussion

1. Interprétation des résultats

1.1. Signification fonctionnelle potentielle

Les résultats des études présentées dans cette thèse mettent en évidence les patrons modulateurs uniques induits par PMv, PMd et SMA des deux hémisphères sur les efférences de M1 vers les muscles de l'avant-bras et de la main. Ces influences prémotrices contrastantes semblent fournir un substrat à travers lequel chaque aire prémotrice pourrait assumer la fonction spécifique qu'elle semble avoir lors de la prépratation et la production des mouvements de la main. Dans cette optique, la signification fonctionnelle des effets modulateurs observés lors de nos études sera discutée dans cette section.

1.1.1. Fonction potentielle des effets modulateurs de PMv

En premier lieu, nous avons démontré qu'iPMv induit des effets facilitateurs fréquents et puissants sur les efférences de M1. Ces résultats s'intègrent bien à l'idée que PMv joue un rôle important pour la préhension et la manipulation d'objets (Rizzolatti et al., 1988; Fogassi et al., 2001; Davare et al., 2006). Plus particulièrement, PMv semble transformer les caractéristiques physiques d'un objet en configurations précises de la main et des doigts. Les effets facilitateurs puissants provenant d'iPMv, particulièrement sur les efférences de M1 vers les muscles intrinsèques de la main, pourraient donc favoriser l'activation spécifique de ces muscles (Figure 5.1, gauche). Puisqu'une étude précédente a démontré que les muscles intrinsèques de la main sont particulièrement actifs lorsque les doigts sont en contact avec un objet (Brochier et al., 2004), ces effets facilitateurs pourraient être principalement utilisé pour générer les forces nécessaires à la préhension et à la manipulation d'objets. Pour ce qui est des effets plus mixtes et moins puissants d'iPMv sur les muscles extrinsèques de la main et du poignet, ceux-ci pourraient permettre à ces muscles de contribuer de manière plus subtile aux différentes phases du mouvements d'atteinte et de préhension, par exemple en stabilisant la main lors du mouvement d'atteinte ou en ajustant sa position lors de la préhension (Brochier et al., 2004).

Dans l'hémisphère contralatéral à M1, nous avons démontré qu'à l'opposé d'iPMv, cPMv induit des effets inhibiteurs fréquents et puissants sur tous les muscles enregistrés. Une possibilité est que ces effets inhibiteurs favorisent la production de mouvements unilatéraux en diminuant radicalement les efférences motrices de M1 vers les muscles du bras non-impliqué (Figure 5.1., droite). Par exemple, lors d'un mouvement de précision de la main droite, le PMv gauche pourrait inhiber les efférences du M1 contrôlant le bras non-impliqué (c.à-d. le M1 droit) afin de diminuer les efférences motrices de ce dernier vers la main gauche et d'ainsi permettre un mouvement unilatéral de la main droite. Un tel système préviendrait la production de mouvements miroirs lorsqu'un mouvement unilatéral est planifié (Mayston et al., 1999; Beaule et al., 2012).

Manifestement, des études futures devront étudier les interactions entre PMv des deux hémisphères et M1 lors du mouvement afin de tester ces hypothèses. Allant dans ce sens, il a été récemment été démontré chez le macaque que durant la préhension de différents objets, iPMv peut induire des effets facilitateurs et inhibiteurs sur les efférences de M1 vers les muscles de la main et du bras et que ces effets varient selon le type de préhension utilisé pour saisir différents objets (Prabhu et al., 2009). Chez l'humain, Davare et al. (2008) ont également démontré que iPMv module les efférences de M1 vers un muscle intrinsèque de la main de manière différente dépendamment du type de préhension exécuté par le sujet. Une préhension de précision engendrait des effets facilitateurs alors qu'une préhension avec la main entière n'engendrait pas de modulation significative. Dans l'ensemble, ces études s'allient bien avec les résultats démontrant que les neurones au sein d'iPMv déchargent différemment en fonction de l'objet à saisir ou du type de préhension à utiliser (Raos et al., 2006; Umilta et al., 2007). Dans le futur, il sera nécessaire de préciser comment les effets modulateurs d'iPMv et de cPMv évoluent au cours de toutes les phases d'un mouvement d'atteinte et de préhension afin d'élucider pleinement les interactions se produisant entre PMv et M1. Il serait également intéressant de déterminer comment différentes caractéristiques tels que la forme de l'objet, la force générée et le but de l'action influencent les interactions entre PMv et M1. Ce type d'expérience fournirait des informations détaillées quant au rôle spécifique que joue iPMv et cPMv dans la production des mouvements de la main.

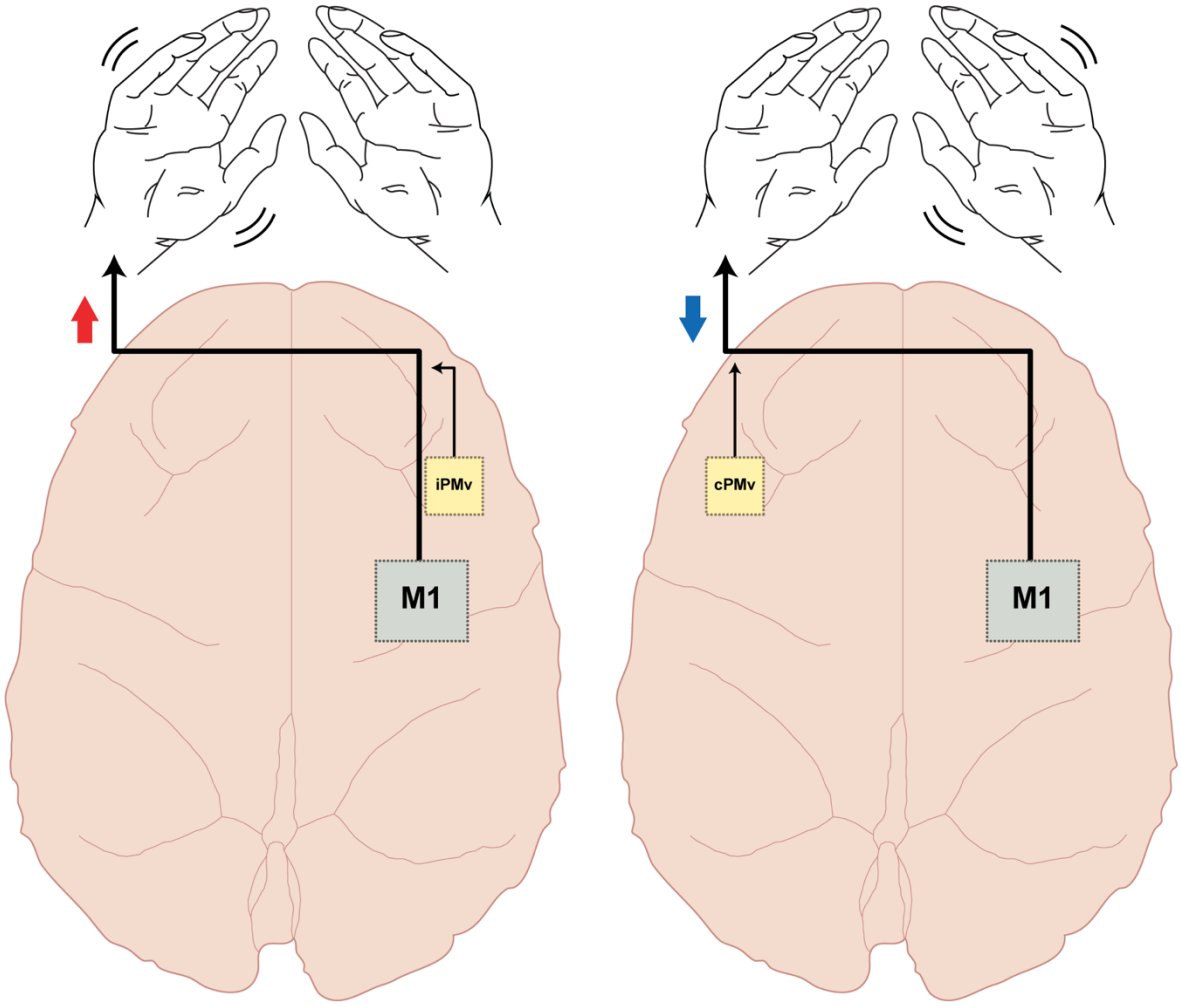


Figure 5.1 *Fonction potentielle des effets modulateurs de iPMv et de cPMv*

À gauche, les effets facilitateurs puissants (flèche rouge) de iPMv sur les efférences de M1 vers les muscles intrinsèques de la main pourraient permettre à iPMv de favoriser l'activation des muscles nécessaires à la production de mouvements de précision avec la main gauche. À droite, les effets inhibiteurs puissants (flèche bleue) de cPMv sur les efférences de M1 vers tous les muscles enregistrés pourraient permettre à cPMv de diminuer l'activation des muscles de la main gauche lors de la production de mouvements unilatéraux avec la main droite afin d'éviter les mouvements miroirs.

1.1.2. Fonction potentielle des effets modulateurs de PMd

En ce qui concerne PMd, nous avons démontré que iPMd induit des effets facilitateurs et inhibiteurs importants sur les efférences de M1 vers tous les muscles testés. Ces résultats s'intègrent bien à l'idée que iPMd joue un rôle crucial dans la préparation et le guidage en temps réel des mouvements d'atteinte et de préhension (Hoshi and Tanji, 2004; Raos et al., 2004; Davare et al., 2006). À travers ce patron modulateur mixte, iPMd pourrait faciliter la préparation et la production de mouvements désirés tout en inhibant celles de mouvements non-désirés (Figure 5.2, gauche). Cette capacité à équilibrer la facilitation et l'inhibition permettrait à iPMd d'avoir un contrôle précis de la configuration du bras et de la main durant les mouvements d'atteinte et de préhension. Tout comme pour PMv, des études futures devront tester ces hypothèses chez le singe éveillé lors de la préparation et l'exécution de mouvements. En particulier, des expériences utilisant des matrices d'électrodes pouvant stimuler et enregistrer plusieurs sites au sein de PMd et de M1 seraient en mesure de démontrer que l'activité d'un neurone de M1 qui décharge lors d'un certain mouvement peut être facilitée par une stimulation dans PMd lors de l'exécution dudit mouvement ou inhibée lors de l'exécution d'un mouvement différent.

Pour ce qui est de l'hémisphère contralatéral, bien que cPMd peut induire des effets inhibiteurs, nous avons démontré que ses effets facilitateurs sur les efférences de M1 sont particulièrement importants, surtout sur les muscles de l'avant-bras. Des effets facilitateurs puissants provenant de l'hémisphère opposé à M1 pourraient pointer vers deux rôles potentiels pour cPMd dans la production des mouvements de la main. Premièrement, les effets facilitateurs de cPMd sur les efférences de M1 pourraient être utiles afin de supporter des mouvements particulièrement complexes de la main ipsilatérale à cPMd (Figure 5.2, milieu). Dans cette optique, M1 aurait besoin de recruter des aires supplémentaires, dont cPMd afin de parvenir à produire des mouvements complexes. Cette hypothèse est soutenue par une étude d'imagerie chez l'humain démontrant que l'activité de cPMd augmente en fonction de la complexité de la tâche lors de l'exécution de mouvements de la main ipsilatérale à cPMd (Sadato et al., 1996). Deuxièmement, une modulation facilitatrice de cPMd sur les efférences de M1 pourrait être utile à la production de mouvements bimanuels complexes nécessitant l'utilisation deux mains de manière indépendante (Figure 5.2, droite). Allant dans ce sens, des études d'enregistrement

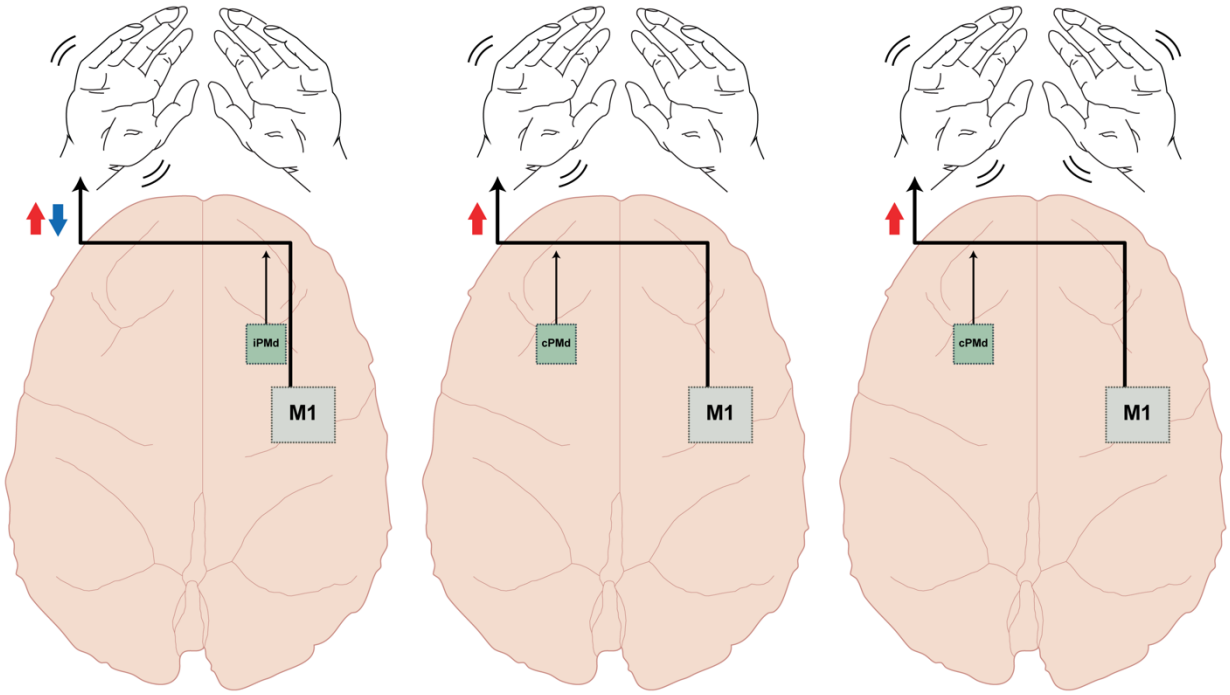


Figure 5.2 *Fonction potentielle des effets modulateurs de iPMd et de cPMd*

À gauche, les effets facilitateurs et inhibiteurs (flèches rouge et bleue) de iPMd sur les efférences de M1 vers tous les muscles testés pourraient permettre à iPMd de favoriser la production de mouvements désirés (facilitation) et diminuer celle de mouvements non-désirés (inhibition) vers la main gauche. Au milieu et à droite, les effets facilitateurs puissants (flèche rouge) de cPMd sur les efférences de M1 vers tous les muscles enregistrés pourraient permettre à cPMd de faciliter l'activation des muscles de la main gauche lors de la production de mouvements complexes avec cette dernière (milieu) ou lors de mouvements bimanuels nécessitant une coordination entre les deux mains (droite).

neural chez le singe (Kermadi et al., 2000) et des études d'imagerie chez l'humain (Meyer-Lindenberg et al., 2002) ont démontré que l'activité de cPMd peut augmenter lors de la production de mouvements bimanuels complexes. De plus, une étude de stimulation pairées chez l'humain a démontré qu'un effet facilitateur de cPMd sur les efférences de M1 pouvait prédire positivement la performance des sujets lors d'un mouvement bimanuel complexe (Liuzzi et al., 2011). De manière intéressante, ce résultat n'était pas observé lors de la performance d'un mouvement bimanuel simple (c.à-d. un mouvement miroir des deux mains). Il est important de noter que les deux rôles potentiels proposés ne sont pas mutuellement exclusifs et afin de les réconcilier, il semble qu'une fonction générale que l'on pourrait imputer à cPMd est de faciliter les efférences de M1 lorsque des mouvements uni ou bimanuels augmentent en complexité. Dans le futur, ces idées devront être testées chez le singe éveillé en étudiant l'impact des effets modulateurs de cPMd lors de différentes phases de mouvements uni et bimanuels plus ou moins complexes.

1.1.3. Fonction potentielle des effets modulateurs de SMA

Finalement, nous avons démontré que iSMA et cSMA induisent des patrons facilitateurs et inhibiteurs assez semblables sur les efférences de M1 aux différents ISIs testés. Toutefois, l'incidence et la puissance des effets facilitateurs étaient généralement plus élevées pour iSMA que pour cSMA. De façon notable, une modulation facilitatrice plus fréquente et plus puissante provenant de l'aire prémotrice ipsilatérale à M1 a également été observée lorsque nous avons comparé iPMv à cPMv et iPMd à cPMd. Ceci porte à croire qu'en général, les aires prémotrices ipsilatérales favorisent davantage la production des mouvements de la main que les aires prémotrices contralatérales. Cet impact facilitateur puissant provenant des aires prémotrices ipsilatérales à M1 s'allie bien avec la notion selon laquelle les mouvements unilatéraux sont principalement contrôlés par l'hémisphère opposé à ces derniers. Toutefois, des études ont démontré que les aires prémotrices contralatérales à M1 sont également recrutées lors de mouvements unilatéraux et ce, particulièrement lorsque la complexité du mouvement à produire augmente (Shibasaki et al., 1993; Sadato et al., 1996). De plus, les aires prémotrices contralatérales semblent fortement impliquées lors de la production de mouvements bimanuels complexes (Sadato et al., 1997; Kermadi et al., 2000; Liuzzi et al., 2011). Ainsi, un constat qui semble découler de ces résultats est que les aires prémotrices contralatérales jouent un rôle de

soutien lors de la production de mouvements qui nécessitent une collaboration entre les deux hémisphères dû à leur complexité. En revanche, les aires prémotrices ipsilatérales joueraient un rôle plus central, favorisant le recrutement des muscles nécessaires lors de tous types de mouvements (c-à-d. simples et complexes). Cette implication considérable des aires prémotrices ipislatérales pourrait expliquer leur impact facilitateur plus puissant sur les efférences de M1. Par ailleurs, il a été démontré que les aires prémotrices possèdent plus de connexions ipsilatérales que contralatérales (Rouiller et al., 1994; Dancause et al., 2006b; Dancause et al., 2007). Ces nombreuses connexions pourraient offrir un substrat soutenant les effets facilitateurs puissants induits par les aires prémotrices ipsilatérales. Allant dans ce sens, l'impact facilitateur observé aux ISIs les plus probablement associés aux voies cortico-corticales directes (ISI1 et ISI2) était particulièrement puissant et ce, pour les trois aires prémotrices étudiées.

Pour revenir à SMA, les patrons modulateurs relativement semblables provenant de iSMA et cSMA aux différents ISIs s'allient bien avec l'idée que cette aire prémotrice est particulièrement associée à la production de mouvements complexes, tels que les mouvements séquentiels unilatéraux ou les mouvements bimanuels (Shima and Tanji, 1998, 2000). Fonctionnellement, ces effets modulateurs similaires pourrait signifier que iSMA et cSMA travaillent de manière synergique afin de coordonner de manière optimale des mouvements complexes (Tanji, 1994; Sadato et al., 1997). De manière intéressante, cPMd semble également fortement impliqué dans la production de mouvements complexes, en particulier les mouvements bimanuels (Kermadi et al., 2000; Liuzzi et al., 2011). Plusieurs études récentes ont souligné l'importance d'un large réseau moteur impliquant plusieurs aires corticales, dont SMA et PMd afin de produire des mouvements bimanuels (Koeneke et al., 2004; Swinnen and Wenderoth, 2004; Grefkes et al., 2008). Ainsi, SMA et PMd pourrait travailler de concert afin de coordonner ce type de mouvements et les nombreuses connexions entre SMA et PMd fourniraient un lien anatomique soutenant cette collaboration (Dum and Strick, 2005). Dans le futur, la contribution de ces deux aires prémotrices lors de mouvements bimanuels chez le singe éveillé pourrait être testée en inactivant SMA et/ou PMd avec un agoniste $GABA_A$ tel que le muscimol lors d'une tâche bimanuelle et observer les changements comportementaux qui en résulte. À travers ce type d'expérience, il serait également important de comparer la contribution de iSMA et de cSMA lors du comportement et de vérifier si les deux SMA sont nécessaires à la préparation et à l'exécution de mouvements séquentiels unilatéraux ou de mouvements bimanuels. Ces études fourniraient

des informations cruciales sur le contrôle cortical du mouvement par SMA qui sont difficiles à obtenir chez l'humain avec des techniques non-invasives tels que la TMS. En dernier lieu, une observation notable de nos études est que SMA des deux hémisphères induit un impact modulateur moins puissant sur les efférences de M1 que PMv et PMd. Une possibilité qui pourrait expliquer ce résultat est que SMA induit ses effets modulateurs de manière moins directe, par exemple, à travers ses nombreuses connexions cortico-corticales avec PMv et PMd (Dum and Strick, 2005). Cette hypothèse pourrait être testée avec une technique telle que la chémogénétique. Bien que généralement employée chez les rongeurs, la chémogénétique à récemment été développé chez le primate et offre la possibilité de manipuler des circuits neuronaux de manière extrêmement précise (Eldridge et al., 2016; Nagai et al., 2016; Galvan et al., 2019; Raper et al., 2019). Par exemple, il serait possible d'inactiver sélectivement les projections de SMA vers PMd ou PMv et d'évaluer, en parallèle, comment les effets modulateurs de SMA sur les efférences de M1 changent suite à cette inactivation. Ceci permettrait de vérifier si les effets modulateurs de SMA dépendent de ses connexions cortico-corticales avec PMd et/ou PMv et d'ainsi mieux définir la fonction de SMA lors la production des mouvements de la main.

1.2. Sites d'interactions potentiels

Nos résultats démontrent que les aires prémotrices de chaque hémisphère modulent les efférences de M1 de manière unique. Ces effets modulateurs distincts provenant de chaque aire prémotrice pourraient être à l'origine de la fonction spécifique que chacune semble avoir pour la production des mouvements de la main. Une question fondamentale qui émerge de ce constat concerne les voies neuronales à travers lesquelles les aires prémotrices peuvent influencer les efférences de M1. Bien que les connexions cortico-corticales offrent, au premier abord, le substrat le plus apparent à l'origine des effets modulateurs observés lors de nos études, les aires prémotrices pourraient également moduler les efférences de M1 par le biais de voies neuronales alternatives. En plus de leurs connexions cortico-corticales avec M1, les aires prémotrices et M1 possèdent des connexions convergentes vers de nombreuses régions sous-corticales. Ainsi, les effets modulateurs observées lors de nos expériences pourraient également se produire au sein de différents sites sous-corticaux. Puisque nos stimulations sont localisées au niveau cortical et que les effets modulateurs sont identifiés au niveau des muscles via des enregistrements EMG, il demeure toutefois difficile d'identifier les sites d'interactions impliqués. Néanmoins, le site

d'interaction le plus probable pour chaque ISI peut être extrapolé par le biais d'études antérieures ayant déterminé le temps de conduction entre les aires motrices corticales.

1.2.1. Temps de conduction entre les aires prémotrices et M1

Plusieurs études examinant les temps de conduction intra et interhémisphérique entre les aires prémotrice et M1 peuvent guider nos hypothèses quant aux sites d'interactions potentiellement impliqués dans nos études. Pour l'hémisphère ipsilatéral, le temps de conduction le plus rapide entre les aires prémotrices et M1 a été estimé à ~1-2 ms (Godschalk et al., 1984; Ghosh and Porter, 1988; Tokuno and Nambu, 2000) alors que pour l'hémisphère contralatéral, il a été estimé à ~2-6 ms (Asanuma and Okuda, 1962; Matsunami and Hamada, 1984; Soteropoulos and Baker, 2007). Par conséquent, ISI1 et ISI2 dans l'hémisphère ipsilatéral ainsi que ISI2.5 et ISI5 dans l'hémisphère contralatéral pourraient être davantage associés aux voies cortico-corticales rapides. En revanche, il semble que le ISI0 laisse peu de temps pour une intégration cortico-corticale et ce, peu importe l'hémisphère. Il est donc possible que les interactions observées au ISI0 se produisent majoritairement au niveau de structures sous-corticales où les projections des aires prémotrices et M1 convergent, tels que le noyau rouge, la formation réticulée ou la moelle épinière (Kuypers and Lawrence, 1967; Monakow et al., 1979; Keizer and Kuypers, 1989; He et al., 1993, 1995; Maier et al., 2002; Borra et al., 2010; Fregosi et al., 2017). Cependant, il est important de noter qu'une stimulation électrique appliquée dans M1 donne lieu à une vague directe de décharge corticospinale (D-wave) suivie de plusieurs vagues indirectes (I-waves) générées par les réseaux locaux de neurones au sein de M1 (Patton and Amassian, 1954; Kernell and Chien-Ping, 1967; Edgley et al., 1997; Maier et al., 2002; Maier et al., 2013). Ainsi, les effets modulateurs observés à l'ISI0 pourraient également être expliqué par l'influence des aires prémotrices sur les I-waves générées quelques millisecondes (~1-3ms) après la stimulation dans M1 (voir section 1.2.2. pour plus de détails) (Cerri et al., 2003; Shimazu et al., 2004; Prabhu et al., 2009). Finalement, il est plus difficile de cerner les sites d'interactions associés aux ISIs les plus longs (ISI4, ISI6 et ISI10 dans l'hémisphère ipsilatéral; ISI10, ISI15 et ISI20 dans l'hémisphère contralatéral) qui pourraient être associés à des voies cortico-corticales lentes, des voies cortico-corticales oligosynaptiques ou encore des voies sous-corticales.

1.2.2. Arguments en faveur d'un site d'interaction au sein de M1

Les sites potentiels d'interactions entre les aires prémotrices et M1 dans le contexte de protocoles de stimulations pairées chez le primate non-humain ont été particulièrement étudiés par Lemon et collègues. Leurs études se sont penchées sur les effets modulateurs de iPMv sur les efférences de M1 et argumentent que ces effets se produisent au niveau cortical, au sein de M1. Plusieurs évidences viennent étayer leur hypothèse. Tout d'abord, lors d'une étude similaire à la nôtre où des protocoles de stimulations pairées entre iPMv et M1 étaient utilisés tandis que l'activité EMG des muscles intrinsèques de la main était enregistrée, Cerri et al. (2003) ont démontré que iPMv commence à moduler significativement les efférences de M1 à partir du ISI1 (ISI testés: ISI0-ISI30). Puisque ce ISI est consistant avec le temps de conduction entre iPMv et M1 (i.e. ~1-2 ms), l'implication d'un site d'interaction au sein de M1 a donc été suggéré tout en soulignant que des preuves plus directes seraient nécessaires afin de soutenir cette proposition. Par la suite, Shimazu et al. (2004) ont utilisé des protocoles de stimulation pairées entre iPMv et M1 tout en enregistrant la voie corticospinale au niveau de la moelle épinière et l'activité intracellulaire de motoneurones innervant les muscles de la main. Sommairement, ils ont démontré que iPMv modulent principalement les I-waves générés par la stimulation dans M1 et que ceux-ci élicitent une augmentation de l'activité des motoneurones enregistrés (Shimazu et al., 2004). Ces effets modulateurs étaient observés à plusieurs ISIs (ISI-0.8-ISI15), dont certains à des délais plus courts que le temps de conduction entre iPMv et M1 (c.à-d. <ISI1). Les auteurs proposent que les effets observés à des ISIs plus courts que 1ms peuvent tout de même se produire au sein de M1 puisque les I-waves quittent le cortex quelques millisecondes (2-4ms) après la stimulation appliquée dans M1. Ceci laisserait donc le temps aux afférences de iPMv d'influencer les neurones de M1, même à des délais plus courts que le temps de conduction entre les deux aires (par exemple ISI0 dans nos études). De manière importante, Shimazu et al. (2004) ont ensuite inactivé M1 à l'aide d'un agoniste $GABA_A$ (muscimol) lors des mêmes protocoles de stimulations pairées et cette inactivation a complètement aboli les effets modulateurs de iPMv précédemment observés (Shimazu et al., 2004). Lors d'une étude subséquente, le même agoniste $GABA_A$ a été injecté dans M1 et les mouvements normalement évoqués par des trains de stimulation appliqués dans iPMv étaient considérablement diminués (Schmidlin et al., 2008). Ces résultats suggèrent que les effets modulateurs de iPMv sur les efférences de M1 de même que son influence générale sur les mouvements de la main dépendent largement de l'intégrité de M1.

Dans leur ensemble, les études de Lemon et collègues fournissent des arguments convaincants en faveur d'un site d'interaction au sein de M1. Des études anatomiques viennent également soutenir leur hypothèse. Il a été démontré que la densité des projections corticospinales de PMv est la plus faible de toutes les aires prémotrices (Dum and Strick, 1991) et que les projections de PMv vers les segments de la moelle épinière innervant les muscles de la main sont peu nombreuses ou même absentes (He et al., 1993; Borra et al., 2010; Morecraft et al., 2019). Ainsi, il semble peu probable que la moelle épinière soit un site d'interaction où PMv peut influencer les efférences de M1. Toutefois, la moelle épinière n'est pas la seule structure sous-corticale où ces interactions pourraient se produire. La contribution potentielle de ces voies alternatives sera discutée dans la prochaine section (voir section 1.2.3.).

Un autre argument intéressant en faveur d'un site d'interaction au sein de M1 peut être avancé à partir d'études anatomiques publiées par notre laboratoire. Celles-ci ont démontré que chaque aire prémotrice est interconnectée préférentiellement à une sous-région spécifique de la représentation de la main de M1 (Dea et al., 2016; Hamadjida et al., 2016). Se basant sur ces résultats, il est tentant de proposer que les effets modulateurs contrastants qui émanent des différentes aires prémotrices (Quessy et al., 2016; Côté et al., 2017; Côté et al., 2019) soient supportés par ces patrons de connexions spécifiques entre les aires prémotrices et M1 (voir Figure 1.1). Dans cette optique, les connexions cortico-corticales uniques reliant les différentes aires prémotrices à M1 fourniraient un substrat neuronal aux résultats fonctionnels décrits dans cette thèse. Chaque sous-région de la représentation de la main de M1 pourrait contenir une population de neurones traitant et intégrant les afférences provenant d'une aire prémotrice en particulier afin de supporter des aspects spécialisés de la fonction motrice de la main. Une telle organisation corticale donnerait lieu à des réseaux parallèles fonctionnels entre les différentes aires prémotrices et M1, et pourrait sous-tendre l'augmentation du répertoire des mouvements de la main chez les primates (Hamadjida et al., 2016). Dans le futur, des expériences supplémentaires pourraient explorer si les interactions fonctionnelles entre les aires prémotrices et M1 mises en évidence dans cette thèse sont organisées en modules comme le suggère nos données anatomiques afin de confirmer cette hypothèse (voir section 2.1.).

1.2.3. Arguments en faveur de sites d'interactions sous-corticaux

Il est important de noter que les arguments présentés par Lemon et collègues en faveur d'un site d'interaction exclusivement localisé au sein de M1 découlent d'expériences testant uniquement les effets modulateurs de PMv sur les efférences de M1. Les sites d'interactions impliqués lors de protocoles de stimulation pairées entre PMd et M1 ou SMA et M1 pourraient être, au moins en partie, différents de ceux de PMv. En effet, PMd et SMA possèdent beaucoup plus de projections corticospinales vers les segments de la moelle épinière innervant les muscles de la main que PMv (He et al., 1993; Maier et al., 2002). Contrairement à PMv, PMd et SMA semblent donc être en mesure de moduler les efférences de M1 au niveau de la moelle épinière. Allant dans ce sens, Maier et al. (2002) ont démontré que SMA et M1 possèdent des projections convergentes vers la moelle épinière sur des interneurones ainsi que des motoneurones innervant les muscles de la main. Ainsi, un site d'interaction au niveau de la moelle épinière semble envisageable en ce qui concerne PMd et SMA. D'autre part, il faut également souligner que PMv, PMd et SMA projettent vers des structures sous-corticales ciblées par M1, telles que le noyau rouge et la formation réticulée (Kuypers and Lawrence, 1967; Monakow et al., 1979; Keizer and Kuypers, 1989; Fregosi et al., 2017). Puisque ces structures sous-corticales projettent à leur tour vers les motoneurones de la moelle épinière innervant les muscles de la main (Holstege et al., 1988; Ralston et al., 1988; Riddle et al., 2009), ces sites d'interactions sont importants à considérer. En fait, il est possible que la variabilité des effets modulateurs que nous avons observée aux différents ISIs lors de nos expériences soit expliquée par le fait que certains ISIs sont préférentiellement associés à des sites d'interactions distincts. Puisque cette variabilité est plus marquée pour les effets modulateurs de PMd et de SMA que ceux de PMv, ceci pourrait pointer vers une implication sous-corticale plus considérable pour ces deux aires prémotrices. Face à ces observations, il serait intéressant d'utiliser des techniques d'inactivation lors de protocoles de stimulations pairées impliquant PMv, PMd et SMA afin de comparer la contribution des voies corticales et sous-corticales provenant de ces différentes aires prémotrices (voir section 1.2.4. pour plus de détails).

1.2.4. Preuves directes des sites d'interactions impliqués

Dans l'intention d'évaluer plus directement la localisation des sites d'interactions impliqués lors des protocoles de stimulations pairées, de futures études pourraient tirer parti de différentes

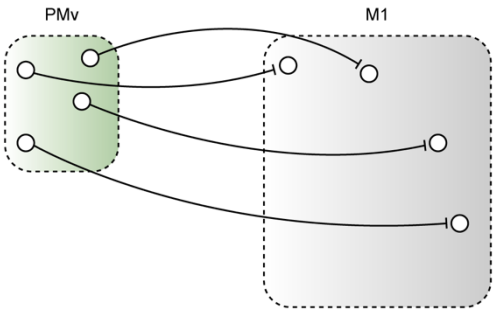
méthodes d'inactivation. Par exemple, afin de déterminer si une structure sous-corticale telle que le noyau rouge ou la formation réticulée est un site d'interaction où les aires prémotrices peuvent moduler les efférences de M1, ces structures pourraient être inactivées localement avec un agoniste $GABA_A$ lors de protocoles de stimulations pairées (Martin et al., 1993; Waitzman et al., 2000). Ceci permettrait d'évaluer si ces voies sous-corticales sont nécessaires à la production des effets modulateurs observés aux différents ISIs. En se fiant aux temps de conduction entre les aires prémotrices et M1 (ipsilatéral : ~1-2ms; contralatéral : ~2-6ms), l'hypothèse la plus plausible est que les effets observés à l'ISI0 soient les plus affectés par ce type d'inactivation sous-corticale. D'autre part, il serait également possible de circonscrire les ISIs particulièrement associés aux voies cortico-corticales en inactivant les connexions entre les aires prémotrices et M1 et en observant l'impact de cette inactivation sur les effets modulateurs aux différents ISIs. De nouvelles techniques telles que l'optogénétique et la chémogénétique seraient particulièrement avantageuses à utiliser pour ce type d'expérience car elles permettent de manipuler des circuits neuronaux de façon très spécifique. Ces techniques se sont récemment développées chez le primate non-humain (Eldridge et al., 2016; Nagai et al., 2016; O'Shea et al., 2018; Yazdan-Shahmorad et al., 2018; Galvan et al., 2019; Raper et al., 2019) et offriraient la possibilité de manipuler sélectivement les neurones d'une aire prémotrice qui projettent vers M1. Par exemple, ces techniques permettraient d'inactiver exclusivement les projections cortico-corticales entre une aire prémotrice donnée et M1 lors de stimulations pairées et d'évaluer leur contribution à la modulation observée aux différents ISIs. En principe, les ISIs associés aux connexions directes entre les aires prémotrices et M1 (ipsilatéral : ISI1 et ISI2; contralatéral : ISI2.5 et ISI5) devraient être les plus influencées par ce type d'inactivation. Globalement, ces études d'inactivation permettraient d'obtenir des preuves plus directes en ce qui concerne la localisation des sites d'interactions corticaux et/ou sous-corticaux impliqués lors de protocoles de stimulations pairées. Ceci aurait un impact substantiel sur l'interprétation des résultats de nos études et de celles réalisées chez l'humain avec la TMS qui démontrent que la modulation observée dépend de l'ISI utilisé.

2. Directions futures

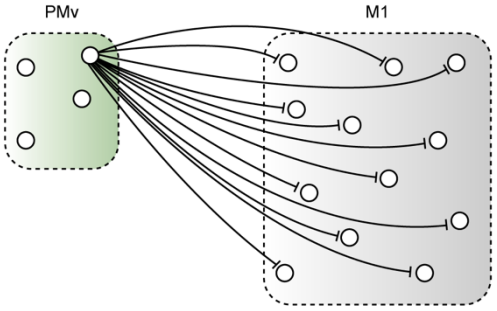
2.1. Topographie des interactions prémotrices-motrices

Bien que nos études aient clairement démontré des profils modulateurs distincts provenant de chaque aire prémotrice sur les efférences de M1 vers les muscles de la main, des études supplémentaires seront nécessaires afin d'obtenir une vision plus détaillée, notamment en ce qui concerne la topographie de ces effets modulateurs. En effet, puisque nous avons utilisé deux microélectrodes (une dans une aire prémotrice et l'autre dans M1) qui étaient déplacées lors de chaque protocole de stimulations pairées, chaque site de stimulation dans les aires prémotrices était associé à un seul site de stimulation dans M1, suivant un ratio de 1 pour 1. En d'autres mots, les effets modulateurs d'un seul site prémoteur sur les efférences d'un seul site dans M1 étaient caractérisés pour chaque protocole (Figure 5.1A). Toutefois, les connexions anatomiques entre les aires prémotrices et M1 sont très vastes. Ainsi, chaque site de stimulation dans les aires prémotrices serait en mesure de moduler l'activité neurale de plusieurs sites dans M1. De plus, différents sites de stimulation dans les aires prémotrices pourraient moduler l'activité neurale d'un même site dans M1 (Godschalk et al., 1984; Tokuno and Nambu, 2000). En somme, le ratio de 1 pour 1 employé dans nos études ne représente pas toute la complexité des interactions possibles entre les aires prémotrices et M1. Face à ce constat, différentes questions émergent. Par exemple, nous pouvons nous demander si un site au sein d'une aire prémotrice donnée engendre des effets semblables ou dissemblables sur différents sites dans M1, ou encore si différents sites à l'intérieur d'une même aire prémotrice engendrent des effets semblables ou dissemblables sur un même site dans M1 (Figure 5.1B et C, respectivement). De plus, en tenant en compte des connexions anatomiques préférentielles entre les aires prémotrices et certaines sous-régions de la représentation de la main de M1 (Dea et al., 2016; Hamadjida et al., 2016), nous pouvons nous demander si les effets modulateurs d'une aire prémotrice donnée sont plus fréquents ou plus puissants sur la sous-région de M1 avec laquelle elle est préférentiellement connectée (Figure 5.1D). En principe, iPMv pourrait avoir des effets modulateurs particulièrement fréquents ou puissants sur la partie rostro-latérale, iPMd sur la partie rostro-médiale et iSMA sur la partie caudo-médiale de la représentation de la main de M1 (voir Figure 1.1). Finalement, nous pourrions également nous interroger sur la divergence et la convergence des effets modulateurs provenant des différentes aires prémotrices sur un même site dans M1. Globalement, répondre à

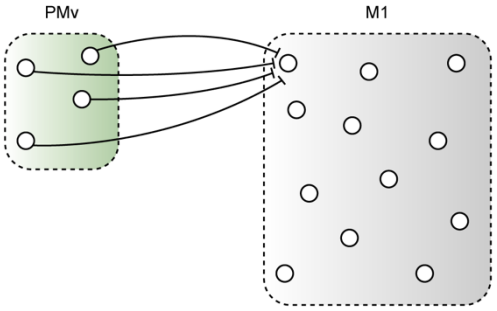
A) Expériences passées: ratio 1 pour 1



B) Expérience future 1



C) Expérience future 2



D) Expérience future 3

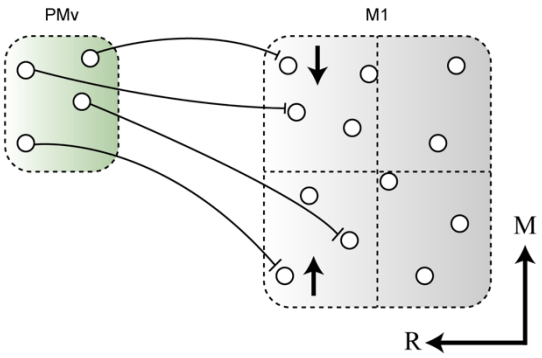


Figure 5.3 *Schémas des expériences passées et futures explorant la topographie des effets modulateurs des aires prémotrices*

A) Schéma représentant l'organisation des expériences présentées dans cette thèse. Dans cet exemple, nous évaluons les effets modulateurs de sites localisés dans PMv (vert) sur des sites localisés dans M1 (gris). Chaque site dans PMv est associé à un seul site dans M1, imposant un ratio de 1 pour 1. **B-D)** Schémas représentant l'organisation d'expériences futures explorant la topographie des effets modulateurs de PMv. En **B)**, les effets modulateurs d'un seul site dans PMv sur plusieurs sites dans M1 sont comparés. En **C)**, les effets modulateurs de plusieurs sites dans PMv sur un seul site dans M1 sont comparés. En **D)**, les effets modulateurs de PMv sur les différentes sous-régions de M1 sont comparés. En se basant sur nos données anatomiques (Dea et al., 2016; Hamadjida et al., 2016), PMv pourrait avoir des effets modulateurs plus fréquents et/ou puissants sur la sous-région rostro-latérale de M1 (flèche vers le haut) que sur la région rostro-médiale (flèche vers le bas). R = Rostral, M = Médial.

ces questions viendrait préciser la topographie des effets modulateurs des aires prémotrices sur les efférences de M1 en spécifiant leur organisation au sein de M1.

Afin d'explorer ces questions, de futures expériences pourraient tirer avantage de matrices de microélectrodes (jusqu'à 100 électrodes) qui peuvent être implantées de manière chronique dans des régions corticales telles que les aires prémotrices et M1. Contrairement à nos expériences aigues lors desquelles nous utilisions deux microélectrodes, l'usage de matrices de microélectrodes implantées dans les aires prémotrices et M1 permettraient d'échantillonner beaucoup plus de sites et ce, à travers des expériences chroniques hebdomadaires. Ultimement, ceci permettrait d'acquérir considérablement plus de données et de tester avec précision la topographie des effets modulateurs des aires prémotrices sur les efférences de M1 vers les muscles de la main. D'un point de vue théorique, ce type d'expériences fournirait des informations détaillées sur les principes organisationnels qui sous-tendent les interactions entre les aires prémotrices et M1. De plus, ces expériences viendraient potentiellement étayer la littérature en fournissant des preuves fonctionnelles aux résultats anatomiques démontrant l'organisation modulaire de M1 (Dea et al., 2016; Hamadjida et al., 2016). S'il est révélé que les aires prémotrices modulent les sous-régions de M1 avec lesquelles elles sont préférentiellement connecté de manière plus fréquente ou puissante, ceci viendrait appuyer l'hypothèse selon laquelle les afférences des différentes aires prémotrices sont traitées séparément à l'intérieur de M1 et intégrées ailleurs dans le système nerveux, par exemple au niveau de la moelle épinière. Ainsi, les projections des aires prémotrices vers M1 pourraient former des réseaux neuronaux parallèles développés au cours de l'évolution afin de traiter des informations motrices distinctes soutenant le répertoire comportemental accru des primates (Hamadjida et al., 2016).

2.2. Protocoles de neurostimulation pour la réhabilitation motrice

En plus de son apport considérable à l'avancement des connaissances fondamentales quant aux interactions corticales qui sous-tendent la production des mouvements de la main chez les primates, nos données pourraient également avoir un impact clinique. Présentement, beaucoup d'intérêt est porté envers le développement des protocoles de neurostimulation chez certaines populations de patients ayant des déficits moteurs, en particulier chez des patients ayant subi un accident vasculaire cérébral (AVC). Les cliniciens élaborant ces protocoles de neurostimulation

se basent sur des données fondamentales telles que les nôtres afin de choisir les meilleures cibles pour ces protocoles ainsi que les paramètres de stimulation les plus efficaces.

Les patients ayant subi un AVC ont souvent des déficits moteurs, notamment une perte de la motricité fine de la main, entraînant une baisse de productivité, d'autonomie et de qualité de vie (Carroll, 1965; Lang and Schieber, 2003; Nowak et al., 2003). Il est bien connu qu'une certaine récupération survient dans les semaines suivant l'AVC dû à la plasticité cérébrale, mais cette récupération est généralement incomplète (Cramer, 2008). De plus, bien que des sessions de réadaptation physique intenses augmentent la récupération motrice, leurs effets sont souvent modestes (Wolf et al., 2006; Winstein et al., 2016). Afin d'atteindre une récupération motrice plus complète, un consensus grandissant soutient qu'il sera nécessaire de développer des thérapies auxiliaires ou des catalyseurs de réadaptation. Parmi ces nouvelles approches, différentes méthodes de stimulation cérébrale présentent des résultats encourageants (Dancause and Nudo, 2011).

2.2.1. Stimulation magnétique transcrânienne répétitive (rTMS)

Des protocoles de stimulation prometteurs présentement testés lors d'études cliniques chez l'humain utilisent une méthode non-invasive appelée stimulation magnétique transcrânienne (TMS) afin de stimuler les neurones d'une région spécifique du cortex avec des champs magnétiques appliqués au-dessus du cuir chevelu. Une des nombreuses variantes de la TMS est la TMS répétitive (rTMS), qui permet d'augmenter ou de réduire l'activité neuronale au sein d'une aire corticale spécifique en stimulant cette dernière de manière répétée. Typiquement, la rTMS à haute fréquence (≥5Hz) augmente la décharge neuronale et la rTMS à basse fréquence (1Hz) la réduit et leurs effets peuvent persister pendant plusieurs minutes après à la stimulation. Dans le cadre de la réhabilitation motrice suite à l'AVC, les protocoles de rTMS ont généralement pour objectif d'accroître l'excitabilité du M1 localisé au sein de l'hémisphère atteint par l'AVC (hémisphère ipsi-lésionnel; iM1), afin de faciliter les efférences motrices de ce dernier vers le membre parétique. Pour ce faire, les protocoles de rTMS testés à ce jour ont principalement tentés de réduire l'activité du M1 contralésionnel (cM1) puisqu'il a été démontré que ce dernier possède une influence inhibitrice sur iM1 (Murase et al., 2004; Duque et al., 2005a). En atténuant l'influence inhibitrice de cM1 sur iM1 avec le rTMS à basse fréquence, ces protocoles avaient

pour but de faciliter les efférences de iM1 vers les muscles du membre affecté. Toutefois, ces études ont produit des résultats mitigés (Takeuchi et al., 2005; Fregni et al., 2006; Bradnam et al., 2012; Talelli et al., 2012). Face à ces conclusions, il est intéressant de noter que d'autres aires corticales, en particulier les aires prémotrices, pourraient offrir des cibles autant sinon plus efficaces que cM1 (Hummel and Cohen, 2006; Grefkes and Fink, 2012). Soutenant cette idée, certaines études ont mis en évidence la contribution positive du PMd contra-lésionnel pour la récupération de la fonction motrice de la main parétique (Lotze et al., 2006; Bestmann et al., 2010; Sankarasubramanian et al., 2017). Dans cette optique, les comparaisons exhaustives entre les différentes aires prémotrices que nous avons menées lors de nos études pourraient être grandement utiles afin de guider le développement de nouveaux protocoles de neurostimulation. Par exemple, dans les cas où l'AVC épargne les zones prémotrices ipsi-lésionnelles, des protocoles de rTMS excitateurs sur iPMv pourraient augmenter ses puissants effets facilitateurs sur iM1 et constituer l'avenue la plus prometteuse pour augmenter les efférences motrices de ce dernier. D'autre part, cibler les aires prémotrices contra-lésionnelles pourrait être un meilleur choix suite à des lésions plus étendues qui génèrent des déficits moteurs sévères. Par exemple, des protocoles de rTMS diminuant l'activité de cPMv et augmentant l'activité de cPMd pourraient atténuer et accentuer leur puissante influence inhibitrice et facilitatrice, respectivement. Allant dans ce sens, il a récemment été rapporté que des protocoles rTMS excitateurs sur cPMd améliore les mouvements d'atteinte chez des patients avec des déficits moteurs majeurs (Sankarasubramanian et al., 2017).

Bien que ces hypothèses soient intrigantes, il n'est toujours pas clair si et comment les effets modulateurs des aires prémotrices changent suite à une lésion de M1 et dans quelle mesure ces changements sont affectés par différents facteurs tels que la taille et l'emplacement de la lésion. Pour être plus efficace, la conception des protocoles de neurostimulation devra tenir compte de ces facteurs et reposer sur une solide compréhension des interactions corticales post-lésionnelles (Hummel et al., 2008; Stagg and Johansen-Berg, 2013). Dû à l'énorme espace de paramétrage du rTMS (par exemple : localisation, stimulation excitatrice ou inhibitrice, intensité, fréquence), déterminer les meilleurs paramètres de stimulation par le biais d'essais cliniques coûteux chez l'humain n'est pas envisageable. Ainsi, des expériences développées chez le primate non-humain tel que le capucin pourrait être particulièrement profitables. De telles expériences permettraient d'étudier de manière efficace les effets de différents paradigmes de

rTMS dans un modèle animal partageant la complexité du réseau moteur cortical humain. En guise d'exemple, l'effet de protocoles de rTMS visant différentes aires prémotrices contra-lésionnelles sur l'activité neurale ipsi-lésionnelle pourrait être évalué avec des enregistrements de neurones ou de potentiel de champ local (LFP) et corrélé avec le comportement de l'animal lors d'une tâche motrice. Ceci permettrait d'identifier la localisation contra-lésionnelle la plus bénéfique à l'augmentation de la fonction motrice de la main tout en révélant les mécanismes neuronaux à travers lesquels le rTMS influence cette récupération. Ensuite, les effets de l'intensité et de la fréquence de la stimulation pourraient être étudiés afin d'optimiser les protocoles ciblant la localisation la plus bénéfique précédemment identifiée. De telles données permettraient de réduire le nombre de protocoles à tester lors d'essais cliniques chez l'humain et de maximiser le potentiel du rTMS comme catalyseur de réhabilitation motrice suite à l'AVC. Bien qu'il reste encore beaucoup à faire, les effets modulateurs distincts provenant de chaque aire prémotrice observés lors des expériences présentées dans cette thèse suggèrent qu'elles pourraient être utilisées lors de protocoles de neurostimulation pour induire un large éventail d'effets et offrir des cibles alternatives et efficaces pour certains patients.

2.2.2. Stimulation pairée associative cortico-corticale (ccPAS)

Une autre avenue qui s'est récemment développée, toujours à l'aide de la TMS, est la stimulation pairée associative cortico-corticale (ccPAS). Contrairement au rTMS qui augmente ou diminue l'activité neuronale d'une seule région corticale, le ccPAS utilise des stimulations simples appliquées au sein de deux aires corticales distinctes. Les deux stimulations sont séparées par un ISI spécifique, et lorsque leur appariement est répété pendant plusieurs minutes, il est suggéré que les connexions entre les deux régions sont renforcées. Cette méthode tente d'exploiter les mécanismes de plasticité associative ou hébienne (Hebb, 1949) qui postulent que si l'activité d'un neurone présynaptique A précède celle d'un neurone postsynaptique B de manière systématique, la connexion A $\rightarrow$ B s'en trouve renforcée. Bi and Poo (1998) ont présenté une version de cette règle de plasticité, connue sous le nom de « spike timing dependent plasticity » (STDP) dans les cellules d'une tranche de l'hippocampe du rat. Lorsque le neurone présynaptique A décharge dans un intervalle de temps compris entre 20 et 30 ms avant la décharge d'un neurone postsynaptique B, la connexion A $\rightarrow$ B est renforcée, un phénomène appelé potentialisation à long-terme (LTP). Au contraire, lorsque le neurone B décharge entre 20 et 30 ms avant que le

neurone A décharge, ceci provoque un affaiblissement de la connexion A → B, appelée dépression à long-terme (LTD). Ainsi, l'idée du ccPAS est de renforcée les connexions entre les aires corticales motrices suite à l'AVC en pairant l'activité d'une aire non affectée par la lésion (par exemple une aire prémotrice) avec l'activité d'une aire affectée (par exemple M1) afin de renforcer les efférences motrices vers le membre parétique. Bien qu'il ait été démontré qu'un phénomène similaire au STDP pouvait être induit par un protocole de ccPAS entre iPMv et M1 chez le sujet sain (Buch et al., 2011; Fiori et al., 2018), aucune étude n'a encore démontré si un tel protocole de stimulation pourrait être bénéfique pour la récupération motrice chez des patients ayant subi un AVC. Tout comme pour la rTMS, le nombre de paramètres à explorer (localisation, fréquence et amplitude de stimulation) est trop vaste pour les tester un à un lors d'essais cliniques chez l'humain. Ainsi, des expériences utilisant le ccPAS chez des primates non-humain ayant subi une lésion seraient fort avantageuses afin d'étudier l'effet de différents paramètres de stimulation ainsi que leurs mécanismes d'action et pourraient aider à l'élaboration d'études cliniques. Toutefois, un point qu'il est important de souligner est qu'étant donné la résolution spatiale limitée du TMS, il est possible que les protocoles de rTMS et de ccPAS ne soient pas en mesure d'induire des effets robustes sur la fonction motrice suite à l'AVC. Puisque que chaque aire prémotrice produit une vaste gamme d'effets modulateurs sur les neurones et les efférences de M1, allant de la facilitation à l'inhibition (Tokuno and Nambu, 2000; Prabhu et al., 2009; Quessy et al., 2016; Côté et al., 2017; Côté et al., 2019), la stimulation d'une aire prémotrice dans son entièreté via la TMS devrait, en principe, potentialiser de manière non-spécifique autant les connexions facilitatrices qu'inhibitrices. Ainsi, la potentialisation des connexions facilitatrices qui seraient susceptible d'aider la récupération motrice pourrait être partiellement ou entièrement annulée par la potentialisation, en parallèle, des connexions inhibitrices. Une façon de contourner ce problème est de s'orienter vers des techniques de stimulation plus invasives.

2.2.3. Stimulations invasives et interfaces cerveau-machine (BMI)

En guise d'alternative au TMS, des protocoles de stimulation utilisant des électrodes intracorticaux ou de surface pourraient entraîner une récupération motrice plus robuste suite à l'AVC puisqu'ils permettent de manipuler l'activité corticale de manière beaucoup plus focale. De manière similaire à la ccPAS, de tels protocoles de stimulation peuvent être utilisés pour renforcer la connectivité entre deux régions corticales en exploitant les mécanismes de plasticité

associative (Hebb, 1949; Bi and Poo, 1998). Plusieurs études ont commencé à explorer cette possibilité en convertissant les potentiels d'action d'un neurone localisé dans un site cortical A en stimuli envoyés dans un site cortical B afin de renforcer les connexions physiologiques entre le site A et le site B. Cette technique nommée stimulation activité-dépendante peut être appliquée via une interface cerveau-machine (BMI) qui permet à un dispositif externe (par exemple une puce informatique fixée sur la tête) d'enregistrer et de stimuler les électrodes de manière indépendante. Des expériences utilisant cette technique ont notamment démontré qu'il est possible d'induire de la plasticité entre différents sites au sein de M1 chez le macaque (Jackson et al., 2006) et entre le cortex prémoteur et le cortex sensoriel primaire (S1) chez le rongeur (Guggenmos et al., 2013). Les résultats de cette dernière expérience soutiennent qu'une stimulation déclenchée dans S1 quelques millisecondes après qu'un potentiel d'action ait été enregistré dans le cortex prémoteur facilite la récupération motrice suite à une lésion traumatique dans M1. En extrapolant ces résultats, il semble qu'une stratégie impliquant la stimulation d'une région endommagée (par exemple M1) basée sur l'activité d'une région épargnée (par exemple une aire prémotrice) pourrait favoriser la récupération motrice suite à l'AVC. Bien qu'une telle stimulation activité-dépendante possède des avantages, l'un des inconvénients est la difficulté d'enregistrer, à long-terme, un signal fort au sein du site A afin de pouvoir déclencher la stimulation dans le site B. Ceci est particulièrement problématique pour les applications cliniques, puisque l'interface doit demeurer efficace tout au long de la vie du patient. Ainsi, d'autres travaux se sont tournés vers des paradigmes de stimulation pairées qui évitent la nécessité d'enregistrer un signal neural (Rebesco and Miller, 2011; Seeman et al., 2017). De manière intéressante, Seeman et al. (2017) ont démontré que le délai optimal pour potentialiser les connexions entre S1 et M1 avec des stimulations pairées chez le macaque est compris entre 10 et 30 ms, ce qui est consistant avec les délais de la STDP (Bi and Poo, 1998). À nouveau, on peut s'imaginer que de tels protocoles de stimulation pairées pourraient permettre de renforcer les connexions entre les aires prémotrices et M1 et d'ainsi augmenter la récupération motrice suite à l'AVC.

Qu'il s'agisse de protocoles de stimulation activité-dépendante ou de stimulations pairées, ces techniques invasives fournissent une alternative attrayante au TMS car elles permettent de favoriser certaines connexions par rapport à d'autres (facilitatrices par rapport à inhibitrices). Par exemple, on pourrait implanter une matrice d'électrode intracorticale (Utah array) dans une aire

prémotrice et une autre dans M1 et sonder la nature (c.-à-d. facilitatrice ou inhibitrice) des connexions entre toutes les paires d'électrodes possibles durant l'exécution d'un mouvement donné. En principe, il serait ensuite possible, via des mécanismes similaires à la LTP, de faciliter ledit mouvement en renforçant exclusivement les connexions facilitatrices entre l'aire prémotrice et M1 avec des protocoles de stimulation contrôlés par BMI visant des paires d'électrodes spécifiques. Un tel système permettrait également de diminuer certaines connexions inutiles ou néfastes au mouvement à travers des protocoles de stimulations induisant de la LTD. En injectant des stimulations hautement spécifiques, ce type d'intervention pourrait améliorer l'efficacité de la récupération motrice suite à l'AVC. De plus, étant donné la portabilité des BMI, un autre avantage est la possibilité d'utiliser ces techniques de stimulations invasives en dehors du laboratoire de manière continue, ce qui pourrait aider à produire des changements à plus long-terme que la TMS. Traditionnellement, le contrôle neural du mouvement a été étudié chez des animaux exerçant des tâches répétitives et hautement entraînées et ce, dans un espace de travail restreint. La mesure dans laquelle les résultats obtenus dans ces conditions sont valables pour les mouvements naturels et non contraints demeure relativement inexplorée. Avec un système invasif de stimulations contrôlé par BMI, il serait possible de renforcer des connexions spécifiques de manière prolongée alors que l'animal se déplace librement dans sa cage et exécute l'ensemble des mouvements compris dans son répertoire comportemental. De telles expériences fourniraient des informations extrêmement utiles au développement de neuroprothèses ayant pour but de réhabiliter les mouvements quotidiens de patients ayant subi un AVC. Bien que ces techniques invasives possèdent un énorme potentiel, il demeure que la complexité engendrée par toutes les combinaisons possibles d'électrodes et tous les paramètres de stimulation entraîne des défis computationnels assez importants qui devront être adressés avant que ce type de système soit considéré comme une avenue clinique intéressante.

En effet, la plupart des études testant des protocoles de stimulation invasifs contrôlés par BMI ont utiliser une quantité limitée d'électrodes. Bien que fournissant des preuves de concept, ces expériences laisse ouverte la question de comment cette approche peut être généralisée pour induire de la plasticité multi-électrodes de manière spécifique et fonctionnelle. Chercher de manière efficace les patrons de stimulations spatio-temporels optimaux à partir d'un grand nombre d'électrodes est une tâche complexe à accomplir en raison de l'explosion combinatoire qui en découle. Une recherche exhaustive manuelle de ces paramètres est impraticable, surtout si

l'on prévoit utiliser ces stimulations dans un contexte clinique. Ainsi, le développement d'algorithmes d'apprentissage automatique est une avenue prometteuse pour optimiser les stimulations multi-électrodes (Lajoie et al., 2017; Rao, 2019). Ce type d'algorithme pourrait être utiliser afin de trouver les meilleurs protocoles de stimulation possibles pour atteindre une connectivité désirée entre certains sites corticaux ou pour faciliter un mouvement ciblé. Bien qu'ils n'aient pas encore été validés sur des modèles animaux suite à l'AVC, ces algorithmes pourraient avoir un impact clinique considérable. Dans cette perspective, les résultats présentés dans cette thèse sont importants à considérer lors du développement de ces algorithmes afin de prendre en compte toute la complexité propre au système moteur cortical.

En résumé, en plus d'augmenter nos connaissances fondamentales sur la nature des interactions se produisant entre les aires prémotrices et M1, nos résultats pourraient avoir un impact sur les thérapies visant à améliorer le contrôle moteur suite à l'AVC en fournissant des informations physiologiques cruciales au développement de techniques de neurostimulation non-invasives et invasives.

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